Presynaptic Group II mGluR Inhibition of Short-Term Depression in the Medial Perforant Path of the Dentate Gyrus In Vitro

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Kilbride, John, Anthony M. Rush, Michael J. Rowan, and Roger Anwyl. Presynaptic group II mGluR inhibition of short-term depression in the medial perforant path of the dentate gyrus in vitro. J Neurophysiol 85: 2509–2515, 2001. Inhibition of short-term plasticity by activation of presynaptic group II metabotropic glutamate receptors (group II mGluR) was investigated in the medial perforant path of the dentate gyrus in the hippocampus in vitro. Brief trains of stimulation (10 stimuli at 1–200 Hz) evoked short-term depression of field excitatory postsynaptic potentials (EPSPs). The steady-state level of depression, measured after 10 stimuli, was frequency dependent, increasing between 1 and 200 Hz. Activation of group II mGluR by the selective agonist LY354740 did not alter short-term depression evoked by frequencies up to 10 Hz, but did inhibit short-term depression evoked at higher frequencies in a frequency- and concentration-dependent manner. The time-averaged postsynaptic response (EPSP per unit time) was found to increase linearly with frequency up to ~20 Hz. At higher frequencies, the response plateaued, thereby becoming independent of frequency. Frequencies above this were differentiated only during the transient postsynaptic response that accompanies changes in firing rates. Activation of presynaptically located group II mGluR increased the frequency at which the EPSP per unit time plateaued up to ~30–50 Hz.

INTRODUCTION

Short-term depression is an activity-dependent reduction in synaptic efficacy that has been observed at a wide variety of synapses in the CNS (reviewed by Zucker 1989). This depression is widely believed to result from a reduction in the amount of neurotransmitter released from presynaptic terminals. Short-term depression in cortical synapses has been shown to operate as an automatic gain control mechanism that unlike inhibitory or adaptive mechanisms has the advantage of being input specific (Abbott et al. 1997; Tsodyks and Markram 1997). As such, afferents that are very active are handled at a lower gain, while the overall strength of the synapse, determined postsynaptically, remains at the same level.

Presynaptically located G-protein-coupled autoreceptors are widespread in the CNS, and many studies have shown that activation of such presynaptic receptors results in inhibition of transmitter release at very low test frequencies at which no short-term plasticity occurs (Anwyl 1999; Thompson et al. 1993; Wu and Saggau 1997). However, there have been few studies investigating the effect of presynaptic autoreceptors at higher stimulation frequencies at which short-term plasticity is evoked, although it has recently been shown that activation of presynaptic GABA_B, ACh, and adenosine receptors results in a reduction of the extent of short-term depression evoked by high-frequency stimulation (Brenowitz et al. 1998; Isaacson and Hille 1997; Tsodyks and Markram 1997; Varela et al. 1997).

In the present study, the effects of activation of group II mGluR on short-term plasticity was investigated at medial perforant path–granule cell synapses in the dentate gyrus that contain a high density of presynaptically located group II mGluRs (Shigemoto et al. 1997). Previous studies have shown that at low stimulation test frequencies, activation of group II mGluRs with selective agonists such as LY354740 (Kilbride et al. 1998) or (2S,1R,2R,3R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) (Brown and Reyman 1994; Huang et al. 1999; Kilbride et al. 1998; Macek et al. 1996; Yokai et al. 1996) results in a concentration-dependent reversible depression of the excitatory synaptic transmission in the medial perforant path.

METHODS

Slice preparation

All experiments were carried out on hippocampal slices obtained from male Wistar rats (50–70 g; BioResources Unit, Trinity College, Dublin, Ireland). Slices were obtained as described previously (Kilbride et al. 1998). Briefly, the brain was rapidly removed after decapitation and placed in cold (5°C) oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 26 NaHCO_3, 1.25 NaH_2PO_4, 2.5 KCl, 2 MgSO_4, 2 CaCl_2, and 10 glucose. Hippocampal slices (350 μM) were cut using a Campden vibroslice (Campden Group Instruments, London) and transferred immediately to an incubation chamber where they were maintained at room temperature, for a period of at least 60 min. Single slices were then transferred to a submersion type recording chamber perfused with ACSF at 30–31°C.

Electrophysiology

Field excitatory postsynaptic potentials (EPSPs) were recorded using standard glass electrodes filled with ACSF. Field EPSPs were generated by a Master 8 eight-channel, programmable pulse generator (A.M.P.I., Jerusalem, Israel) driven by pCLAMP 6 software (Axon Instruments, Foster City, CA) on an IBM-compatible PC. Stimulation pulses (0.1 ms duration) were delivered via a bipolar insulated tungsten wire electrode, adjusted to give about 30% of the maximal response (~1 mV). The EPSPs were amplified by a Grass P16 microelectrode DC amplifier (Grass Instruments, Quincy, MA), converted from A/D form (Axon Instruments, Digidata 1200) before

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being stored on a Dell dimension 466 PC for subsequent off-line data processing. Trains of 10–20 stimuli were delivered to the medial perforant path. Where stated, the frequency at which the stimulation was being delivered was instantaneously increased after 10 stimuli, either by a fixed percentage (50 or 100%) or by a fixed increment (5 Hz) of the initial stimulation frequency (see Abbott et al. 1997).

Data analysis and statistics

Summarized results are expressed as EPSP mean amplitude ± SE. The amplitude of each EPSP in a train was expressed as a percentage of the first control EPSP, and the “normalized” EPSP (%) was calculated by normalizing every value in a particular experimental protocol to the first EPSP amplitude in that protocol. Data were analyzed using Student’s paired t-test, and repeated measures ANOVA, at the 5% level of significance.

The rate of onset of depression in the medial perforant path was calculated using Graphpad Prism software and verified on Jandel Sigmaplot software. The summary data from seven experiments at a range of frequencies between 0.5 and 200 Hz were fitted with a one-phase exponential decay curve using the following equation: \( Y = \text{Span} \ast \exp(-K \ast x) + \text{plateau} \). Starts at span and decays to plateau with a rate constant \( K \). The time constant of decay (\( \tau \)) was calculated as \( 1/K \).

The reduction in short-term depression by activation of group II mGluR was estimated as Data points were constructed by expressing the steady-state EPSP amplitude in LY354740 as a percentage of the steady-state EPSP amplitude in control conditions, over a range of frequencies. The arrows in Fig. 3 show the threshold frequency of significant LY354740-induced attenuation of the short-term depression. This was established by comparing at each frequency, the steady-state EPSP amplitude in LY354740, as a percentage of control, with the test frequency (0.0166 Hz), using Student’s t-test, at the 5% level of significance (mean ± SE, \( n = 4–11 \)).

Compounds

LY354740 (\((+)\)-2-aminobicyclo[3.1.0]hexane-2–6-dicarboxylic acid), which was a generous gift from Eli Lily (Indianapolis, IN), was dissolved in freshly made NaOH (0.1 N) and was added directly to the perfusate after establishing a steady baseline. This was accompanied by no observable change in the pH of the ACSF.

RESULTS

Brief trains of stimuli at 1–200 Hz evoke a reversible depression of synaptic transmission

Field EPSPs remained at a stable amplitude when evoked at the test frequency of 0.0166 Hz. However, trains of 10–20 stimuli delivered at frequencies of 1–200 Hz resulted in a reversible depression of the amplitude of EPSPs (Fig. 1A). The depression occurred rapidly during the initial five stimuli and then stabilized at a steady-state level during the subsequent five stimuli. Trains were limited to 10–20 stimuli to minimize a long-term form of synaptic depression (Dittman and Regehr 1998; Galarreta and Hestrin 1998). The extent of steady-state depression increased as the frequency was increased between 1 and 200 Hz (Fig. 1B). For example the steady-state EPSP amplitude at 1, 50, and 200 Hz was 74.8 ± 2.9% (mean ± SE, \( n = 5 \)), 32.4 ± 2.3% (\( n = 9 \)), and 8.4 ± 2.2% (\( n = 5 \)) of the initial EPSP amplitude respectively (ANOVA, \( P < 0.05 \)). The rate of onset of short-term depression also increased as the frequency was raised, having time constants of decay of 813, 210, and 17 ms for 1, 10, and 50 Hz, respectively (\( n = 5–9 \)). The rate of onset and extent of short-term depression were found to be independent of stimulation intensity as test EPSPs that varied between 0.4 and 2.2 mV elicited almost identical results (data not shown).

Short-term depression at frequencies above 10 Hz is inhibited by activation of group II mGluRs

LY354740 is a recently synthesized potent and specific agonist at group II mGluRs (Monn et al. 1997; Schoepp et al. 1997). Previous electrophysiological studies carried out in this laboratory have shown that LY354740 has a potent presynaptic reversible inhibitory action on EPSPs evoked at the test frequency in the medial perforant path, with an IC_{50} of ~100 nM, and maximal inhibition of 80% at ~5 μM (Kilbride et al. 1998). To investigate whether the activation of group II mGluRs by LY354740 modulates short-term plasticity, brief
trains of 10 stimuli were applied at frequencies of 1–200 Hz after the inhibition of the test EPSP by LY354740 had attained equilibrium (20 min perfusion). No desensitization of the inhibition generated by LY354740 was observed within the time period for which LY354740 was applied, usually up to 40 min.

At frequencies up to 10 Hz, short-term depression was unaffected by either an intermediate (100 nM) or a high (5 μM) dose of LY354740. For example, at 1 Hz, when the data were normalized to the first EPSP in each condition, no significant difference in the normalized steady-state EPSP was observed in the presence of a low or high concentrations of LY354740. Thus the normalized steady-state EPSP amplitude was 74.8 ± 2.9% (n = 4), 72.3 ± 2.0% (n = 4), and 71.3 ± 5.2% (n = 4) in control, 100 nM, and 5 μM LY354740, respectively; values were not significantly different (Fig. 2A).

At frequencies >10 Hz, short-term depression was inhibited by the activation of group II mGluRs with LY354740, and strong facilitation was often observed. For example, at 50 Hz, the short-term depression that occurred in control was strongly reduced in both the intermediate (100 nM) and high (5 μM) dose of LY354740, and facilitation occurred in the high dose of LY354740 (Fig. 2B). The normalized steady-state depressed amplitude of the EPSP was 32.4 ± 2.3% (n = 9), 51.1 ± 1.8% (n = 7), and 91.6 ± 4.6% (n = 10) in control, 100 nM, and 5 μM LY354740, respectively (ANOVA, P < 0.05).

The concentration and frequency dependency of the group II mGluR–induced inhibition of short-term depression is shown in Fig. 3A, which displays the amplitude of the steady-state EPSP in LY354740 (100 nM and 5 μM) as a percentage of the steady-state control EPSP between 0.0166 and 200 Hz. Above a certain threshold frequency, the amplitude of the steady-state EPSP amplitude in LY354740, relative to control, was increased as the stimulation frequency became higher, demonstrating inhibition of short-term depression. The threshold frequency at which LY354740 reduced short-term depression was 50 Hz for 100 nM LY354740 and 20 Hz for 5 μM LY354740. Interestingly at 100 Hz, the attenuation of short-term depression was so pronounced that the steady-state EPSP amplitude in LY354740 was actually larger than in control conditions.

**Time-averaged postsynaptic response becomes independent of frequency for input rates greater than ~20 Hz.**

An estimate of the amount of transmitter released per unit time can be established by plotting the steady-state EPSP amplitude times frequency as a function of stimulation frequency (Abbott et al. 1997; Curtis and Eccles 1960; Richards 1972; Tsodyks and Markram 1997). In these studies, the mobilization of transmitter became rate limiting above a certain limiting frequency. A rate limitation of the mobilization of transmitter was also found to occur in the present study. Thus the EPSP amplitude per unit time was approximately linearly related to frequency up to ~20 Hz but approached a constant value at higher frequencies (Fig. 3B). The modulation of short-term depression by activation of group II mGluRs is further revealed in the curves of the normalized EPSP per unit time to frequency, plotted in the presence and absence of LY354740 (Fig. 3B). LY354740 shifted the curve upward from control in a concentration-dependent manner due to a shift to higher frequency of the point at which the normalized EPSP per unit time became independent of frequency (Fig. 3B). Thus in
LY354740 (5 µM), the limiting frequency was shifted upward to 50 Hz. Short-term depression at frequencies above 10 Hz is reduced by lowering extracellular Ca²⁺ into the presynaptic terminals. Previous studies have suggested that the inhibitory action of presynaptic mGluRs on EPSPs elicited at low frequencies is a result of a reduced influx Ca²⁺ into the presynaptic terminals. To investigate whether the presynaptic action of LY354740 may be due to a reduction of Ca²⁺ influx into the presynaptic terminal, the effect of low extracellular concentrations of Ca²⁺ were investigated on short-term depression.

At the test frequency of 0.0166 Hz, the steady-state EPSP amplitude was reduced as the level of extracellular Ca²⁺ was lowered. In 1.6, 1.2, and 0.8 mM Ca²⁺ the test (0.0166 Hz) EPSP amplitude was measured as being 63.9 ± 16.8% (n = 3), 49.8 ± 10.3% (n = 4), and 29.6 ± 8.6% (n = 5) of control (2 mM; ANOVA, P < 0.05). Trains of stimuli at low frequency, up to ~10 Hz, were not inhibited by the low Ca²⁺ media. For example, at 1 Hz the curves relating the short-term depression in 2 mM (control), 1.6, 1.2, and 0.8 mM Ca²⁺ were investigated on short-term depression.
mM Ca$^{2+}$ overlap, and moreover, the normalized steady-state EPSP amplitudes were not significantly different: 70.6 ± 2.4%, (n = 8), 76.6 ± 2.4%, (n = 3), 77.9 ± 3.0% (n = 6), and 69.9 ± 18.1% (n = 4), respectively (Fig. 4A). However, high-frequency stimulation in low extracellular Ca$^{2+}$ resulted in a reduction of the short-term depression. For example at 50 Hz, the reduction in the level of extracellular Ca$^{2+}$ was accompanied by a slowing down of the rate of onset of depression and a reduction in the extent of short-term depression. The normalized steady-state EPSP amplitudes were 33.0 ± 2.0% (n = 11), 43.8 ± 2.3% (n = 3), 56.1 ± 4.8% (n = 7), and 126.2 ± 32.1% (n = 4) in 2.0, 1.6, 1.2, and 0.8 mM Ca$^{2+}$ (P < 0.05, Fig. 4B). The curves relating the normalized EPSP per unit time to frequency were shifted upward as the limiting frequency was extended from 10 to 20 Hz in control to over 50 Hz in 0.8 mM Ca$^{2+}$ (Fig. 4C).

Effect of an instantaneous increase in the stimulation frequency in control and following activation of group II mGluR

In the absence of short-term depression, the response to fixed percentage frequency increments would increase linearly as a function of the initial input frequency. Short-term depression at excitatory synapses in the cortex has previously been shown to exert a type of synaptic gain control, equalizing the response to fixed percentage frequency changes at higher frequencies while amplifying the response to lower frequencies (Abbott et al. 1997). The effects of instantaneous frequency changes were investigated in the present study on the medial perforant path to dentate granule cell synapse, and modulation of the effects following the activation of group II mGluR also studied. The frequency was increased instantaneously by a fixed percentage (50 or 100%) after an initial 10 stimuli at frequencies between 0.5 and 50 Hz, and a further 10 stimuli
delivered at the new rate. Measurements were made of the effects of the instantaneous increase in frequency on both the EPSP and EPSP per unit time.

An instantaneous fixed percentage increase of 100% in the frequency of stimulation following the initial 10 stimuli at frequencies of 0.5–50 Hz resulted in further depression of the EPSP amplitude to a new steady-state amplitude, reached after a further 5–10 stimuli, with the extent of the additional depression being dependent on the frequency (Fig. 5A). In contrast to this further depression of the EPSP amplitude on an instantaneous rise in frequency, the EPSP per unit time was actually initially increased by the instantaneous frequency rise. The increase in the EPSP per unit time in response to fixed percentage increases was approximately proportional to the magnitude of the frequency change for frequencies over ~20 Hz, i.e., the time-averaged postsynaptic response to a 100% change in firing rate was almost twice that of a 50% change (Fig. 6A). Furthermore the absolute increase in the time-averaged postsynaptic response to a 100% increase in the afferent firing rate did not differ significantly at frequencies above 10 Hz (Fig. 6A).

**FIG. 6.** The transient EPSP change to fixed percentage (50 or 100%) instantaneous frequency changes and its modulation following activation of group II mGluRs, plotted over a full range of frequencies. A: absolute increases in EPSP per unit time associated with an instantaneous increase in firing rate of either 50% (○) or 100% (●) plotted as a function of the initial stimulation frequency. Each point shows the mean and SE (n = 4–7). B: the increase in the EPSP per unit time after a 100% increase in firing rate is reduced by LY354740 (5 μM; ○) only at frequencies of 25 Hz and below. Each point shows the mean and SE (n = 7–14).

Activation of group II mGluRs was found to reduce the responsiveness to instantaneous frequency changes of 100% at frequencies lower than 25 Hz, but not at higher frequencies. Thus a significant reduction in the absolute increase in the EPSP per unit time from control occurred in the presence of LY354740 with instantaneous increases from 0.05 to 1 Hz, 5 to 10 Hz, 10 to 20 Hz, and 25 to 50 Hz, but not 40 to 80 Hz or 50 to 100 Hz (Figs. 5B and 6B).

**DISCUSSION**

**Short-term depression and frequency of stimulation**

The results of this study have shown that short-term plasticity in the medial perforant path of the dentate gyrus is a context-dependent and dynamic phenomenon. Under normal physiological conditions, the predominant response to an increased frequency of stimulation is short-term depression, whereas when the probability of release is lowered, for example, during the activation of presynaptic group II mGluRs, facilitation becomes more prominent, especially at higher frequencies. Both the rate of onset and extent of short-term depression were found to increase with stimulation frequency.

The effects of short-term depression on the limiting frequency in the medial perforant path of the dentate gyrus are in broad agreement with similar studies in the cortex (Abbott et al. 1997; Markram et al. 1998; Tsodyks and Markram 1997; Varela et al. 1997). Thus in the present study, the limiting frequency for transmitter mobilization was ~20 Hz, similar to that found in the visual cortex (10 Hz) (Abbott et al. 1997) and somatosensory cortex (10–25 Hz) (Tsodyks and Markram 1997), although it should be noted that previous studies made use of a high Ca²⁺ to Mg²⁺ ratio, which has the effect of increasing the rate of short-term depression and consequently lowering the limiting frequency. The limiting frequency of ~20 Hz found in the present study represents the upper ceiling to rate coding. At lower frequencies the time-averaged EPSP increases with frequency and therefore a neuron can decipher the information encoded by the rate of presynaptic input. Above the limiting frequency, the time-averaged EPSP is independent of the presynaptic firing rate, and therefore rate coding is limited.

**Inhibition of short-term depression by activation of presynaptic group II mGluRs**

The results of the present study show that activation of presynaptic group II mGluRs results in a concentration- and frequency-dependent inhibition of the observed short-term depression. A similar phenomenon has been observed following activation of other presynaptic G-protein–coupled receptors, e.g., muscarinic ACh, GABA_B, and adenosine A₁ receptors (Barnes-Davies and Forsythe 1995; Brenowitz et al. 1998; Isaacson and Hille 1997; Lev-Tov and Pinc 1992; Pennartz and Lopes da Silva 1994; Shen and Horn 1996; Tsodyks and Markram 1997; Varela et al. 1997). In this study, the inhibition of short-term depression by activation of group II mGluRs only became significant above 50 and 20 Hz for concentrations of 100 nM and 5 μM LY354740, respectively. These results reaffirm that the effects of activation of autoreceptors are context dependent and dynamic. For example, although an autoreceptor may be inhibitory during low-frequency stimulation, facilitatory pro-
cesses are unmasked and become increasingly significant during high-frequency stimulation.

Activation of group II mGluR was found to extend the linear range of the curves relating the EPSP per unit time to frequency, which is consistent with its action on short-term depression. Thus the limiting was extended from ~20 to ~50 Hz. The response to temporal changes in input rate was also consistent with a shift toward a synapse displaying less synaptic depression. Under control conditions, small fluctuations in slowly firing afferents are handled at a higher gain; however, group II mGluR activation equalizes the postsynaptic response to small fluctuations on slow and rapidly firing afferents. In contrast there is less equality in the response to fixed percentage changes in input rates above ~20 Hz, and consequently, the curves relating the absolute increase in the EPSP per unit time to frequency, for fixed percentage rate changes in control and LY354740, converge at ~50 Hz. In effect this means that the ability of the postsynaptic cell to respond to fractional depression becomes less when afferents are handled at a higher gain; however, group II mGluR activation equalizes the postsynaptic response to small fluctuations on slow and rapidly firing afferents. Potential for multiple mechanisms, phenomena and algorithms for synaptic plasticity at single synapses. Neuropharmacology 37: 489–500, 1998.

Lowering extracellular Ca\(^{2+}\) had a very similar action to that of activation of group II mGluR, with short-term depression being relieved at relatively high, but not low, frequencies, and the limiting frequency being shifted to a higher value as extracellular Ca\(^{2+}\) was lowered. It is therefore likely that inhibition of Ca\(^{2+}\) influx underlies the mechanisms of activation of group II mGluR. The exact mechanism may involve an inhibition of Ca\(^{2+}\) channels (Takahashi et al. 1996; Wu and Saggau 1995), a facilitation of K\(^+\) channels (Sladeczek et al. 1993), or the action may be at a site downstream from the site of Ca\(^{2+}\) entry (Hille 1994).

In conclusion, the results of the present studies in the medial perforant path of the dentate gyrus demonstrate that short-term depression acts as an automatic gain control system, amplifying small fluctuations on slowly firing afferents (Abbott et al. 1997). At low firing rates, when depression is mild, temporal integration predominates, while at higher firing rates at which depression becomes more prominent, temporal coherence becomes more important, possibly acting as a complement to the limited rate coding mechanism (Tsodyks and Markram 1997). Activation of group II mGluRs results in a reduction of short-term depression and reduces the temporal changes on slowly firing afferents in preference to more rapidly firing ones.

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REFERENCES


