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Blocking polysynaptic inhibition via opioid receptor activation isolates excitatory synaptic currents without triggering epileptiform activity in organotypic hippocampal slices

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Abstract

The abundance of synaptic connectivity in the cultured hippocampal slice preparation allows measurements of the unitary excitatory connection between pairs of pyramidal neurons using simultaneous presynaptic and postysynaptic intracellular recordings. However, the useful yield of these recordings can be greatly reduced by the presence of polysynaptic inhibition that occludes the measurement of the monosynaptic excitatory postsynaptic current (EPSC). We have found that the traditional method of eliminating contaminating synaptic inhibition with GABA receptor antagonists is of limited usefulness because the recurrent excitatory connections in organotypic slices cause epileptiform bursting in the absence of inhibitory function. This bursting obscures EPSCs to an even greater extent than the normally occurring polysynaptic inhibitory transmission. Here, we report a new method for isolating monosynaptic EPSCs using the mu-opioid agonist peptide DAMGO to reduce polysynaptic inhibition during these recordings. Activation of mu-opioid receptors is known to hyperpolarize inhibitory neurons. We found that DAMGO application reduces the amplitude and frequency of polysynaptic inhibition, allowing isolation of the excitatory connection between the two neurons being recorded. Furthermore, because inhibitory function is not completely eliminated by DAMGO application, epileptiform bursting very rarely develops. Therefore, the use of DAMGO to prevent polysynaptic inhibition without causing epileptiform bursting provides a useful tool to substantially increase the yield of experiments measuring the unitary excitatory connection between pyramidal neurons in the cultured hippocampal slice preparation.

Keywords: Hippocampus; Synaptic transmission; Synaptic inhibition; CA3; Opioid; DAMGO; Epilepsy

1. Introduction

The cultured hippocampal slice preparation is advantageous for studies of synaptic function and plasticity because of increased synaptic connectivity compared to an acute slice. The probability of an excitatory connection occurring between two pyramidal neurons in CA3 is about an order of magnitude greater in the cultured slice than in the acute slice (Malinow, 1991; Pavlidis and Madison, 1999). This increased probability of connection makes feasible studies that examine the unitary excitatory connection between pairs of individual neurons. With this technique, the response of a single postsynaptic neuron to neurotransmitter release by a single presynaptic neuron can be recorded. This readily allows direct recording of the amplitude and failure rate of the unitary connection between neurons, permitting recordings of phenomena such as silent (NMDA only) synapses, which would be impossible in measurements of the summed response to many presynaptic inputs (Montgomery et al., 2001).

A problem that arises when trying to make electrophysiological recordings of the monosynaptic EPSC between two neurons, however, is that the abundance of synaptic connections in the slice can result in the activation of interneurons, even though only a single presynaptic pyramidal neuron is being stimulated. This results in large polysynaptic inhibitory postsynaptic currents (IPSCs) in the postsynaptic neuron, occluding the measurement of the monosynaptic EPSC. Simply blocking inhibitory transmission in the slice by application of GABA receptor antagonists, such as bicuculline,

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is not a viable solution because removal of inhibition from such a recurrent network results in epileptiform bursting, which precludes any study of normal synaptic function and plasticity. Attempting to avoid epileptiform activity during the application of GABA receptor antagonists by simultaneously applying drugs that globally reduce excitatory synaptic activity in the slice, such as the adenosine receptor agonist 2-Cl adenosine, have the significant drawback that they alter the function of excitatory neurotransmission itself (Tancredi et al., 1998), which is the focus of the recording.

To overcome these problems, we applied the mu-opioid receptor agonist DAMGO to cultured slices. Since mu-opioid receptors are specifically localized on interneurons in the hippocampus (Drake and Milner, 1999; Drake and Milner, 2002) and result in membrane hyperpolarization (Madison and Nicoll, 1988; Svoboda and Lupica, 1998; Svoboda et al., 1999; Wimpey and Chavkin, 1991), we reasoned that activation of these receptors might cause individual EPSPs onto interneurons to be subthreshold for action potential activation. At the same time, inhibitory interneurons could still be recruited to prevent network bursting because the onset of increasing excitatory activity would result in summated EPSPs onto interneurons that could trigger their action potential firing. Using paired whole cell recordings of CA3 pyramidal neurons; we found that DAMGO could in fact block polysynaptic IPSCs without triggering epileptiform activity, allowing for the isolation of EPSCs.

2. Methods

2.1. Preparation of slices

Interface cultures of hippocampal slices were prepared as described (Stoppini et al., 1991) using 7-day-old Sprague–Dawley rats. Hippocampi were dissected in minimum essential medium (MEM, Gibco Invitrogen) with 15 mM HEPES and 10 mM Tris-buffer (Gibco). Four hundred micrometer slices were cut using a Stoelting Tissue Slicer (Stoelting Co.). Slices were cultured on Millicell CM culture plate inserts (Millipore). The culture medium consisted of 50% MEM, 25% Hanks balanced salt solution (HBSS), and 25% horse serum, with 12.5 mM HEPES buffer and penicillin (100 U/ml)/streptomycin (100 μg/ml) (all from Gibco). Cultures were maintained in 5% CO_2, at 37 °C for 3 days and then at 34 °C for the remaining culture period. Slices were recorded from after 7–12 days in culture.

2.2. Electrophysiological recordings

Two simultaneous whole-cell recordings were performed in area CA3. During recording, slices were immersed in artificial cerebrospinal fluid (ACSF) at room temperature, containing (in mM) 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 Na_2HPO_4, 26.2 $NaHCO_3$, 11 glucose, perfused at a rate of 2 ml/min. Pyramidal cells in area CA3 were visualized by infrared DIC microscopy. The internal solution consisted of (in mM): 120 K gluconate (presynaptic cell) or Cs gluconate (postsynaptic cell), 40 HEPES, 5 $MgCl_2$, 0.3 NaGTP, 2 NaATP, 5 QX314 (postsynaptic cell only), pH 7.2 with KOH or CsOH. Presynaptic neurons were held in standard current clamp mode and postsynaptic neurons were held in voltage clamp mode using a MultiClamp 700A (Axon Instruments, Union City). Presynaptic action potentials were induced by 20 ms current pulse (typically 20–50 pA). Postsynaptic currents in response to presynaptic action potential firing were elicited at 0.1 Hz throughout the duration of each recording. In addition to acquiring sweeps of each action potential-induced event at 10 kHz (using a Digidata 1322A, Axon), the output signals from the amplifier were also sent to a second digitizer (MiniDigi 1A, Axon) and sampled continuously at 1 kHz to monitor for epileptiform activity that could be missed in the record of individual episodic data sweeps. The mu-opioid agonist peptide Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAMGO) was kept frozen at 2.5 mM in water and aliquots were diluted to the final working concentration on the day of each experiment.

3. Results

3.1. Polysynaptic IPSCs can occlude the measurement of monosynaptic EPSCs

Polysynaptic IPSCs are frequently seen in paired whole cell recordings from CA3 pyramidal neurons in organotypic slices. While approximately 50% of paired recordings yield an excitatory connection (Montgomery et al., 2001), the yield of useable recordings can be greatly decreased by the presence of polysynaptic IPSCs that occlude the measurement of the EPSC (Fig. 1A). Polysynaptic IPSCs often occur in over half of all paired recordings in a given slice preparation. Thus the overall probability of getting a recording from a pair of neurons in which a monosynaptic EPSC is present, and an occluding polysynaptic IPSC is not present can be very low. Therefore, it is of particular interest to find ways to eliminate polysynaptic IPSCs and increase the yield of useful measurements of monosynaptic EPSCs in paired whole cell recordings from organotypic hippocampal slices.

3.2. GABA receptor antagonists block polysynaptic IPSCs but trigger epileptiform bursting

The most direct way to remove inhibition from the recordings is to add antagonists of the ionotropic GABA receptors such as bicuculline. However, there are numerous recurrent connections between the excitatory neurons in the cultured slices, so complete removal of GABA inhibition from the slices by bicuculline invariably results in epileptiform bursting (Figs. 1B, 4A). This bursting not only frequently occludes measurement of EPSCs in response to the evoked action potentials, but the presynaptic bursts of action potentials cor-