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CO-INDUCTION OF LONG-TERM POTENTIATION AND LONG-TERM DEPRESSION AT A CENTRAL SYNAPSE IN THE LEECH

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Abstract

Most studies of long-term potentiation (LTP) have focused on potentiation induced by the activation of postsynaptic NMDA receptors (NMDARs). However, it is now apparent that NMDAR-dependent signaling processes are not the only form of LTP operating in the brain (Malenka and Bear, 2004). Previously, we have observed that LTP in leech central synapses made by the touch mechanosensory neurons onto the S interneuron was NMDAR-independent (Burrell and Sahley, 2004). Here we examine the cellular mechanisms mediating T-to-S (T→S) LTP and find that its induction requires activation of metabotropic glutamate receptors (mGluRs), voltage-dependent Ca^{2+} channels (VDCCs) and protein kinase C (PKC). Surprisingly, whenever LTP was pharmacologically inhibited, long-term depression (LTD) was observed at the tetanized synapse, indicating that LTP and LTD were activated at the same time in the same synaptic pathway. This co-induction of LTP and LTD likely plays an important role in activity-dependent regulation of synaptic transmission.

Keywords

metabotropic glutamate receptor; voltage-dependent Ca^{2+} channel; protein kinase C; neuroplasticity; invertebrate

NMDAR-dependent long-term potentiation (LTP) and long-term depression (LTD) are thought to be critical cellular substrates for mediating learning and memory because their initiation requires coincident activity in both the pre- and postsynaptic neurons (activity dependence) and the resulting changes are restricted to the co-activated synapses (synapse specificity). However, it is now clear that other molecules can perform coincidence-detection in place of NMDARs for both LTP and LTD (Malenka and Bear, 2004; Anwyl, 2006). This heterogeneity in cellular mechanisms mediating LTP and LTD, along with the structural complexity of the vertebrate brain, complicates efforts to determine the functional contribution of synaptic changes to learning-related changes in behavior. The medicinal leech (*Hirudo medicinalis*) has a number of properties that make it a useful model for studies of LTP and LTD. Most neurons in the leech CNS are large and easily visualized and there are far fewer neurons in the leech CNS (~400 neurons/ganglion with 21 body ganglia plus the head and tail ganglia (Muller et al., 1981)) compared to a mammalian brain. Therefore, it is possible to record

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from the same, identifiable neuron from one animal to the next and to link changes in a given neuron to a specific behavioral function (Burrell and Sahley, 2005; Kristan et al., 2005). Furthermore, the cellular and molecular properties between leech and vertebrates neurons are highly conserved (Burrell and Sahley, 2001), so discoveries about neural function in invertebrates are relevant to understanding processes in vertebrate neurons.

LTP and LTD have been observed in two different synaptic connections in the leech CNS; those made by the touch (T) sensitive cells onto the S interneuron (S-cell) and by pressure (P) sensitive onto the same S-cell. The S-cell is thought to be critical for certain types of learning in the leech (Modney et al., 1997; Burrell et al., 2003). LTP in the P→S synapse is NMDAR-dependent, synapse-specific, and expressed postsynaptically (Burrell and Sahley, 2004). At the T→S synapse (Fig. 1A), which is the focus of this paper, tetanic stimulation simultaneously induces homosynaptic LTP (homLTP) in the tetanized synapse and heterosynaptic LTD (hetLTD) in the non-tetanized synapse (Fig. 1B; also see Burrell and Sahley, 2004). This pattern of homLTP and hetLTD (synapses consisting of different presynaptic cells, but the same postsynaptic target) has been observed in the CA1 (Lynch et al., 1977), CA3 (Kosub et al., 2005) and dentate gyrus (Abraham and Goddard, 1983) regions of the hippocampus, the amygdala (Royer and Pare, 2003) and the visual cortex (Tsumoto and Suda, 1979). HomLTP at the T→S synapse is NMDAR-independent while T→S hetLTD is NMDAR-dependent (Burrell and Sahley, 2004). In this study, we examined underlying homLTP at the T→S synapse and discovered that inhibition of homLTP uncovered LTD in the same synapse that was apparently initiated in parallel with LTP in the tetanized pathway.

To test the signaling pathways that mediate T→S LTP, individual ganglia were dissected from 3g leeches obtained from a commercial supplier (Leeches USA Ltd.) and aintained in pond water (0.5 g/1 L H_2O Hirudo salt from Leeches USA Ltd.) at 18°C with a 12h:12h light/dark cycle. Dissections and recordings were carried out in normal leech saline (in mM: 115 NaCl, 4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$ and 10 HEPES). Following dissection, ganglia were placed in a recording chamber under constant perfusion. Intracellular recordings from identified T- and S-cells were made using glass sharp microelectrodes connected to a bridge amplifier (BA-1; National Precision Instruments). A detailed protocol for inducing homLTP and hetLTD in the T→S synapse are described in Burrell and Sahley (2004). In brief, unitary excitatory postsynaptic potentials (EPSPs) were elicited in the S-cell by stimulation of the presynaptic neuron (the T-cell) prior to (pre-test) and 1 hr after (post-test) tetanic stimulation of the dorsal posterior nerve root. Ten tetani were delivered at 10sec intervals, with each tetanus consisting of five stimuli delivered at 25Hz (STG 1004 Programmable Stimulator; Multichannel Systems). Drugs were applied after the pre-test for 10mins with the tetanizing stimuli applied at the end of this period (all drugs were obtained from Sigma). T→S synaptic transmission was tested at the tetanized synapse (T^+) and the non-tetanized connection (T^-). Only two recordings (pre- and post-tetanus) were made because chronic (>10–15min) recordings of the S-cell damage the interneuron (Burrell and Sahley, 2004).

In normal saline, tetanic stimulation elicited homLTP in the tetanized T→S synapse and hetLTD in the non-tetanized connection (Fig. 1B and 2), replicating the results obtained in Burrell and Sahley (2004). The T→S synapse is glutamatergic (Li and Burrell, 2006) and since T→S LTP is NMDAR-independent, the potential involvement of mGluRs was investigated. HomLTP was blocked in ganglia treated with 1 mM alpha-methyl-4-carboxyphenylglycine (MCPG, an antagonist of mGluR1, 2 and 5) during tetanic stimulation. MCPG did not block hetLTD, indicating that mGluRs contribute only to T→S homLTP and not to hetLTD (Fig. 2A), which has already been shown to be NMDAR-dependent (Burrell and Sahley, 2004). Surprisingly, homosynaptic LTD (homLTD) was observed in the tetanized T→S synapses (T^+) of the MCPG-treated ganglia in addition to the hetLTD at the non-tetanized synapse (T^-; Fig. 2A). Apparently, the same tetanus that elicits mGluR-dependent homLTP in the

tetanized T→S synapse simultaneously initiates homLTD in the same synapse that can only be observed when homLTP is blocked.

If mGluRs are required for T→S LTP, then they likely act in concert with other molecules to form a coincidence detection system that is an alternative to the NMDAR. One possible combination is mGluRs plus VDCCs in which mGluRs detect synaptic input and VDCCs detect depolarization. To test this hypothesis, 20μM nimodipine, an antagonist of L-type Ca^{2+} channels, was applied during tetanus. As with blockade of mGluRs, nimodipine prevented T→S homLTP and LTD was observed at both the tetanized and non-tetanized pathways (Fig. 2B).

One potential intracellular target of mGluRs and VDCCs is PKC. Group I mGluRs activate phospholipase C that leads to the production of DAG, which along with Ca^{2+} influx from the VDCCs, activates PKC (De Blasi et al., 2001). To test the involvement of PKC during T→S LTP, the membrane permeable PKC antagonist bisindolylmaleimide (BIS, 10μM) was applied during the tetanus. Again, LTP in the tetanized pathway was blocked and LTD was observed at both the tetanized and non-tetanized synapses (Fig. 2C).

In many synapses LTP is initiated by NMDAR activation, which acts as a coincidence detector of pre- and postsynaptic activity. However, there are a number of forms of LTP and LTD, including T→S LTP, where a combination of mGluRs and VDCCs act as a coincidence detection system (Schrader et al., 2004; Bender et al., 2006; Codazzi et al., 2006). In the case of the T→S synapse, it is likely that mGluR activation and Ca^{2+} influx via the VDCCs converge on PKC, given that group I mGluRs activate phospholipase C that leads to the production of DAG, which along with Ca^{2+} influx from the VDCCs, activates PKC (De Blasi et al., 2001). However, MCPG is not a selective mGluR antagonist, so the involvement of group 1 mGluRs has yet to be confirmed. The precise site of LTP in this polysynaptic pathway is not known and therefore it is impossible to say whether T→S LTP manifests as an increase in presynaptic neurotransmitter release, an increase in postsynaptic glutamate receptor density/function or a combination of both pre- and postsynaptic mechanisms.

A surprising result from these experiments is that whenever homLTP was prevented at the T→S synapse, homLTD was observed. The depression observed in the various drug-treated synapses was due to the tetanic stimulation and not to the application of the drugs themselves given that MCPG, BIS, nimodipine and BAPTA treatments without tetanization had no effect on synaptic transmission (Fig. 2). It was already known that the same tetanizing stimulus that induced homLTP in the active T→S synapse simultaneously induced hetLTD in the inactive T→S synapse (same postsynaptic S-cell, different presynaptic T-cells; see Fig. 1B, 2 and Burrell and Sahley, 2004). The data presented here indicate that homLTD is also induced at the active, tetanized synapse, but is masked by homLTP. The signaling mechanisms mediating T→S homLTD at the tetanized synapse are not known, but there are a number of observations that suggest that this LTD is NMDAR-dependent. First, hetLTD produced by the same tetanic stimulation and observed at the same time, but at a different T→S connection, is NMDAR-dependent (Burrell and Sahley 2004). Second, one would expect that if the homLTD was NMDAR-dependent, than the magnitude of the homLTP would increase in the presence of drugs that blocked NMDAR function. This, in fact, was observed by Burrell and Sahley (2004; see Fig. 7A) when LTP was induced in the presence of the NMDAR antagonist APV. Third, T→S homLTD induced by low frequency stimulation (450 stimuli at 1Hz) is blocked by the NMDAR antagonists, APV and MK-801 (unpublished data).

It has been known that certain activity patterns could simultaneously induce multiple forms of synaptic plasticity at different synapses given that similar patterns of homLTP and hetLTD have been observed (Lynch et al., 1977; Tsumoto and Suda, 1979; Abraham and Goddard,

1983; Bradler and Barrionuevo, 1990; Martinez et al., 2002; Royer and Pare, 2003; Kosub et al., 2005). However, recent findings, including those presented here, now indicate that co-induction of LTP and LTD can actually occur in the same synapse (O'connor et al., 2005; Bender et al., 2006; Nevian and Sakmann, 2006; Sjostrom et al., 2007; Tzounopoulos et al., 2007). One potential function of this co-induction of LTP and LTD is that it contributes to spike-timing dependent plasticity (STDP). In STDP different patterns of coincident pre- and postsynaptic activity elicit either LTP or LTD (Dan and Poo, 2004). It has been suggested that STDP utilizes two independent processes of synaptic plasticity, one mediating potentiation and the other depression, and that the relative level of activation of these two processes determines whether LTP or LTD is produced (Bender et al., 2006; Nevian and Sackman, 2006; Tzounopoulos et al., 2007). Alternatively, co-induction of LTP and LTD at the same synapse may allow for a kind of metaplasticity, (Abraham & Bear 1996) where modulation of one form of synaptic plasticity alters the magnitude of the other. For example, modulation that results in a decrease in homLTD would be expected to cause the level of the co-induced homLTP to increase.

The data presented here demonstrate the complexities of understanding the cellular basis of activity-dependent forms of synaptic plasticity. LTP and LTD are often thought to be mediated by NMDAR-dependent processes, but it is increasingly clear that a variety of non-NMDAR-dependent mechanisms exist and are common; examples include LTP and LTD that are mediated by mGluRs, VDCCs, PKC, Ca^{2+} release from intracellular stores, and endocannabinoid receptors (Malenka and Bear, 2004; Anwyl, 2006). In addition, it is now clear that both LTP and LTD processes can be co-activated with the level of synaptic change determined by the interaction of these two processes (Duguid and Sjostrom, 2006). The leech provides a useful model system for examining the cellular mechanism mediating these processes and their functional relevance from the neural circuit to the behavioral level.

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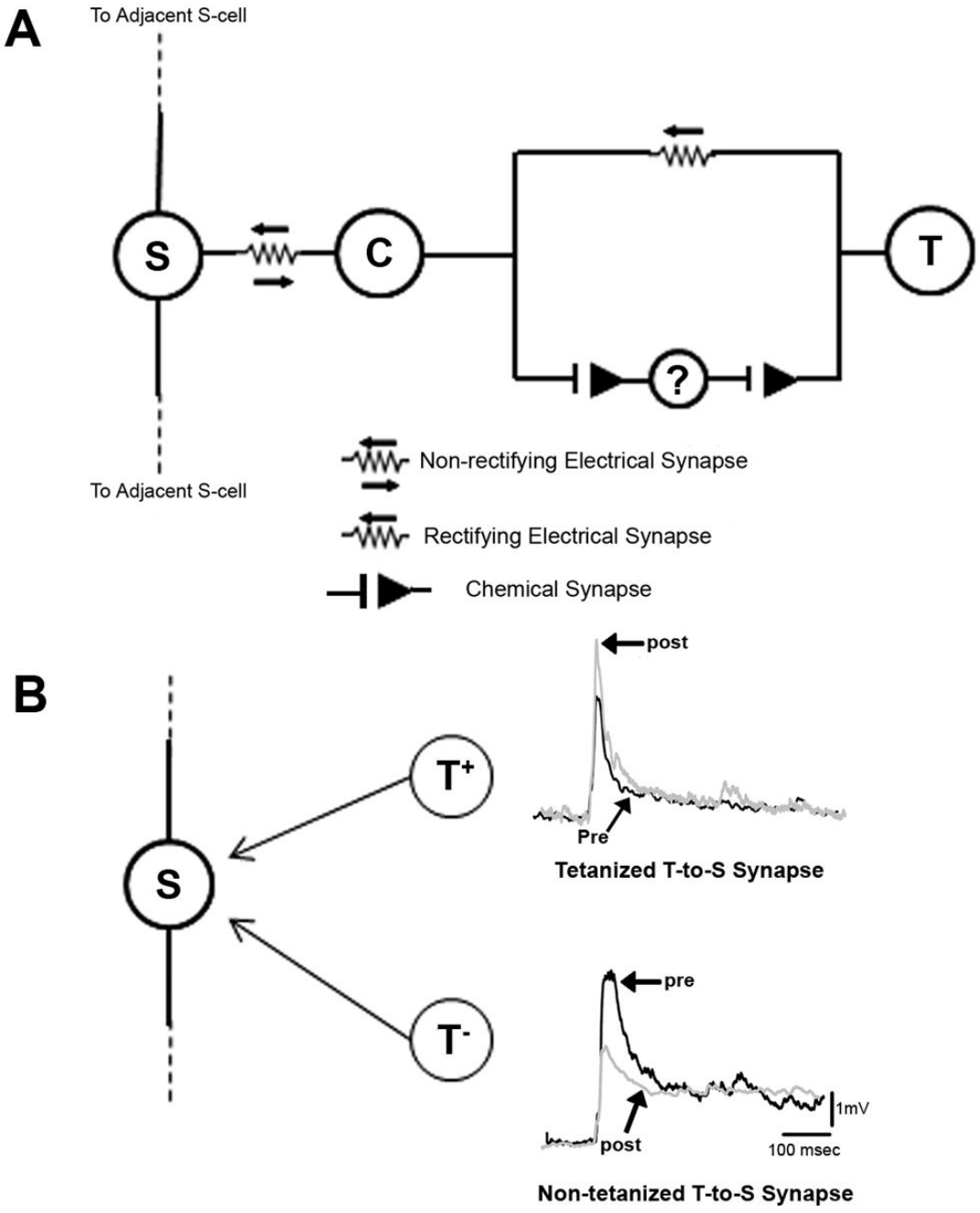

Figure 1.

(A) T→S synaptic circuit. The T→S synapse has both a monosynaptic electrical and polysynaptic chemical (glutamatergic) component (Muller and Scott, 1981; Li and Burrell, 2006). The "?" indicates the unknown neuron(s) that mediate the polysynaptic, chemical component of the T→S synapse. Nearly all synaptic input to the S-cell is routed through the coupling (C) interneuron. The S- and the C-cells are linked by a non-rectifying electrical synapse and the level of electrical coupling is so strong that EPSPs elicited in the C-cell are carried to the S-cell with minimal attenuation or delay, acting as monosynaptic EPSPs (Muller and Scott, 1981). The C-cells are not directly recorded from because they are on the opposite (dorsal) side of the ganglion. (B) Changes in T→S EPSP at the tetanized and non-tetanized

synapse. *Left* Diagrammatic representation of convergent inputs by the two T-cells, the tetanized (T^+) and non-tetanized (T^-), onto a single postsynaptic S-cell. *Right* Traces labeled "pre" were recorded prior to tetanic stimulation and those labeled "post" were recorded 60min after tetanus. Tetanization of the DP nerve elicited homLTP in the tetanized T→S synapse and simultaneously elicited hetLTD in the non-tetanized T→S synapse (same postsynaptic S-cell, different presynaptic T-cells).

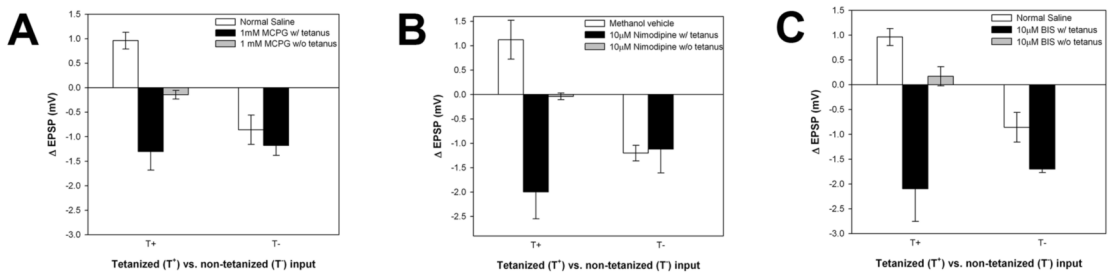

Figure 2.

Cellular mechanisms of T→S LTP. All data are expressed as a ΔEPSP between an initial pretest and a posttest 60min later. T^+ represents the tetanized T→S synapse, while T^- represents the non-tetanized pathway. Under control conditions (saline (N=8) or methanol vehicle (N=4)), tetanization induces homLTP in the T^+ pathway and hetLTD in the T^- pathway. (A) Treatment with the mGluR antagonist, MCPG, blocked homLTP and homLTD was observed at the T^+ synapse instead (N=7). MCPG did not affect hetLTD at T^- and MCPG applied without tetanus did not alter synaptic transmission (N=4). (B) Treatment with the VDCC blocker, nimodipine, blocked homLTP and homLTD was observed at the T^+ synapse instead (N=4). Nimodipine did not affect hetLTD at T^- and nimodipine applied without tetanus did not alter synaptic transmission (N=3). (C) Treatment with the PKC antagonist, bisindolylmaleimide (BIS), blocked homLTP and homLTD was observed at the T^+ synapse instead (N=4). BIS did not affect hetLTD at T^- and BIS applied without tetanus did not alter synaptic transmission (N=4).