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Activity-Dependent β-Adrenergic Modulation of Low Frequency Stimulation Induced LTP in the Hippocampal CA1 Region

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Summary

β-Adrenergic receptor activation has a central role in the enhancement of memory formation that occurs during heightened states of emotional arousal. Although β-adrenergic receptor activation may enhance memory formation by modulating long-term potentiation (LTP), a candidate synaptic mechanism involved in memory formation, the cellular basis of this modulation is not fully understood. Here, we report that, in the CA1 region of the hippocampus, β-adrenergic receptor activation selectively enables the induction of LTP during long trains of 5 Hz synaptic stimulation. Protein phosphatase inhibitors mimic the effects of β-adrenergic receptor activation on 5 Hz stimulation–induced LTP, suggesting that activation of noradrenergic systems during emotional arousal may enhance memory formation by inhibiting protein phosphatases that normally oppose the induction of LTP.

Introduction

At many excitatory synaptic connections in the brain, the strength of synaptic transmission can be persistently up- or down-regulated, a property thought to play an important role in both activity-dependent developmental processes (Goodman and Shatz, 1993) and in the storage of new information during learning and memory formation (Bliss and Collingridge, 1993). In general, two types of long-lasting changes in synaptic strength can be observed at excitatory synapses, a persistent enhancement of synaptic transmission known as long-term potentiation (LTP) and a persistent depression of synaptic transmission known as long-term depression (LTD). In the CA1 region of the hippocampus, intense activation of N-methyl-D-apartate (NMDA) type glutamate receptors during brief bursts of high frequency synaptic stimulation produces large increases in intracellular Ca^{2+} that induce LTP by activating protein kinases (Bliss and Collingridge, 1993). Several minutes of lower frequency (1–3 Hz) synaptic stimulation elicits less intense NMDA receptor activation and smaller increases in intracellular Ca^{2+}, which induce LTD by activating protein phosphatases (Mulkey and Malenka, 1992; Mulkey et al., 1993, 1994; however, see Neveu and Zucker, 1996).

Beyond this general understanding, however, the molecular details of the processes responsible for LTD and LTP at these synapses are not well understood. For instance, although many features of LTP can be accounted for by the activity of a single protein kinase (Ca^{2+}/calmodulin-dependent kinase II) (Lisman, 1994; Braun and Schulman, 1995; Nicoll and Malenka, 1995), the induction of LTP seems to involve a bewildering array of signaling molecules, including at least six different protein kinases and a number of additional processes (Bliss and Collingridge, 1993). How many of these signaling molecules contribute to LTP is a mystery. In addition to this plethora of signaling molecules, deciphering the molecular events responsible for LTP has been further complicated by the fact that the involvement of many of these molecules in LTP is controversial. For instance, while it is clear that protein kinase A (PKA) has an important role in the late phase of CA1 LTP (3 hr postinduction, Frey et al., 1993), reports differ as to whether PKA also has an important role in the early phase of LTP, with some reports showing that PKA inhibitors block the early phase of LTP (during the first 60 min postinduction) (Musgrave et al., 1993), while others indicate that PKA inhibitors have no effect on the early phase of LTP (Weisskopf et al., 1994). One possibility is that the apparent complexity of the molecular processes underlying LTP is due to the presence of multiple modulatory pathways that influence the induction of LTP (Ben-Ari et al., 1992). Some of the controversy surrounding the role of molecules, like PKA, in the early phase of LTP may be due to the fact that they are actually components of these modulatory pathways and, under different experimental conditions, may be more or less important for the induction of LTP. Indeed, recent findings suggest that PKA activation has a special role in the early phase of LTP as part of a gate-like signaling pathway that enables the induction of LTP by inhibiting protein phosphatases (Blitzer et al., 1995; Lisman, 1994; Iyengar, 1996). Thus, it has been speculated that, for patterns of synaptic stimulation that not only activate the protein kinases responsible for LTP but also activate protein phosphatases, activation of this PKA-dependent molecular gate and subsequent inhibition of protein phosphatases may have a crucial, albeit permissive, role in the induction of LTP (Blitzer et al., 1995).

Importantly, the PKA-dependent gate provides a mechanism whereby modulatory neurotransmitters that increase intracellular cAMP and activate PKA could potently modulate the induction of LTP. However, while previous studies have shown that β-adrenergic receptor activation and subsequent increases in cAMP enhance LTP at excitatory synapses in the dentate gyrus (Sarvey et al., 1989) and CA3 region of the hippocampus (Hopkins and Johnston, 1984; Huang and Kandel, 1996), β-adrenergic receptor activation is not thought to be required for, nor importantly modulate, the induction of LTP by high frequency stimulation in the CA1 region (Sarvey et al., 1989). One reason for this discrepancy may be that by acting through the PKA-dependent gate, β-adrenergic receptor activation might selectively enhance the induction of LTP by weak, near threshold

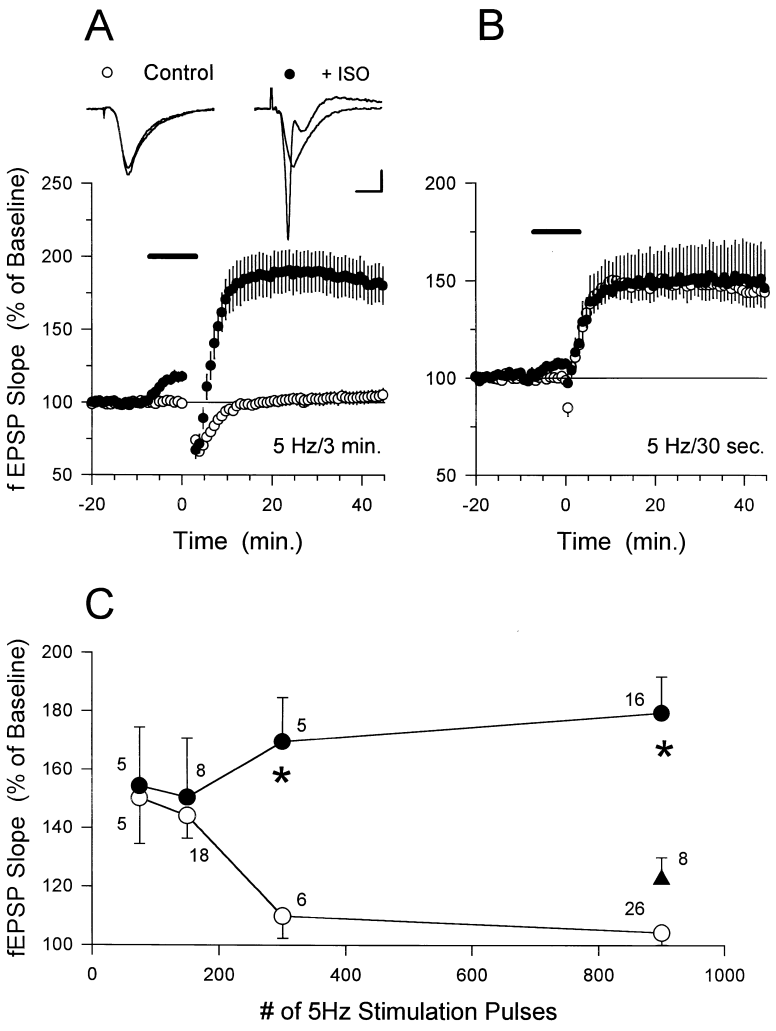

Figure 1. β-Adrenergic Receptor Activation Enhances the Induction of LTP by Long Trains of 5 Hz Stimulation

(A) In control experiments (no ISO), 3 min of 5 Hz stimulation (delivered at time = 0, open symbols, n = 26) had no lasting effect on synaptic transmission (45 min after 5 Hz stimulation, fEPSPs were not significantly different from pre–5 Hz baseline, t(25) = 1.01). However, 3 min of 5 Hz stimulation delivered at the end of a 10 min application of 1.0 μM ISO (indicated by the bar) induced LTP (closed symbols, n = 16, t(15) = 6.36, $p < 0.01$ compared with baseline). The traces are fEPSPs recorded during baseline and 45 min after 5 Hz stimulation in the presence and absence (control) of ISO. Calibration bars are 2.0 mV and 5.0 ms.

(B) A short train of 5 Hz stimulation (30 s) induced LTP in the absence of ISO (open circles, n = 18, t(17) = 5.62, $p < 0.01$ compared with baseline), and this potentiation was not enhanced by ISO (closed circles, n = 8, t(24) = 0.35 compared with 5 Hz for 30 s alone).

(C) Summary of the effects of β-adrenergic receptor activation on changes in synaptic strength induced by different amounts of 5 Hz stimulation. Each point is the mean (±SEM) of responses recorded 45 min after 5 Hz stimulation, number of experiments is indicated next to each point (asterisk indicates $p < 0.01$ compared with 5 Hz stimulation in the absence of ISO). The closed triangle shows the amount of potentiation induced when 3 min of 5 Hz stimulation in ISO was delievered in the presence of 100 μM APV. The small potentiation that remained in the presence of APV was not blocked by nifedipine (fEPSPs were 123.05% ± 3.7% of baseline following 3 min of 5 Hz stimulation in ISO plus APV plus 10 μM nifedipine, n = 5).

patterns of synaptic stimulation where down-regulating protein phosphatase activity may be more important for the induction of LTP. We have thus reinvestigated whether β-adrenergic receptor activation of the cAMP/PKA signaling pathway enhances CA1 LTP by examining whether the β-adrenergic receptor agonist isoproterenol (ISO) enhances the induction of LTP by a low frequency stimulation protocol (3 min of 5 Hz stimulation) that appears to be near threshold for inducing LTP (Mayford et al., 1995).

Results

In hippocampal slices from 3- to 5-week-old animals, 3 min of 5 Hz stimulation delivered to the Schaffer collateral/commissural fibers in the CA1 region had no long-term effects on synaptic transmission (Figure 1A, field excitatory postsynaptic potentials [fEPSPs] were 104.2% ± 4.1% of baseline 45 min after 5 Hz stimulation). Although a 10 min application of ISO (1.0 μM) induced a small enhancement of synaptic transmission (Gereau and Conn, 1994a; however, see Parfitt et al., 1992; Heginbotham and Dunwiddie, 1991), this effect was not long lasting and responses returned to baseline levels following ISO washout (fEPSPs were 105.7% ± 3.1% of baseline 45 min after ISO application, n = 6). However, 5 Hz stimulation delivered in the presence of ISO induced a persistent enhancement of synaptic transmission (Figure 1A; fEPSPs were potentiated to 179.4% ± 12.5% of baseline) that was inhibited by the NMDA receptor antagonist APV (fEPSPs were 122.5% ± 7.3% of baseline after 5 Hz stimulation in ISO plus 100 μM APV, n = 8, t(7) = 3.93, $p < 0.01$ compared with 5 Hz stimulation in ISO). Consistent with the effects of APV, which suggest that the potentiation induced by 5 Hz stimulation in ISO is largely due to the induction of NMDA receptor–dependent LTP, prior induction of saturating levels of LTP using multiple trains of high frequency stimulation (see Experimental Procedures) occluded the potentiation induced by 5 Hz stimulation in ISO (fEPSPs were 104.7% ± 13.6% of baseline after 5 Hz stimulation in ISO was delivered to potentiated synapses, n = 6, not significantly different from baseline, t(5) = 1.07).

By enhancing CA1 pyramidal cell excitability (Heginbotham and Dunwiddie, 1991; Pedarzani and Storm, 1995), β-adrenergic receptor activation could enhance the induction of LTP by indirectly facilitating NMDA receptor activation during 5 Hz stimulation. However, this

simple interpretation is difficult to reconcile with the results from experiments in which we examined the effects of different amounts of 5 Hz stimulation on synaptic strength (Figure 1). Although 1 and 3 min of 5 Hz stimulation had no lasting effects on synaptic strength, shorter trains of 5 Hz stimulation (15–30 s duration) induced modest LTP even in the absence of β-receptor activation (fEPSPs were potentiated to 150.1% ± 15.8% of baseline, n = 10, and 144.1% ± 7.8% of baseline, n = 18, following 15 and 30 s of 5 Hz stimulation, respectively, Figures 1B and 1C). The β-blocker timolol (10 μM) had no effect on the induction of LTP by 30 s of 5 Hz stimulation (fEPSPs were 156.6% ± 14.8% of baseline, n = 4, not significantly different from ISO plus 5 Hz for 30 s of stimulation in the absence of timolol, t(20) = 0.69), but completely blocked the ability of ISO to enhance the induction of LTP by 3 min of 5 Hz stimulation (fEPSPs were 105.9 ± 6.2, n = 5, t(4) = 5.37, $p < 0.01$ compared with ISO plus 5 Hz for 3 min stimulation in the absence of timolol). This suggests that the induction of LTP by 30 s of 5 Hz stimulation is not enabled by small amounts of norepinephrine released from noradrenergic fibers in the slice. Instead, it appears that during 5 Hz stimulation the processes responsible for LTP are initially activated but, as stimulation continues, a second process that opposes LTP is turned on. Interestingly, ISO selectively enhanced the induction of LTP by longer 5 Hz stimulation trains and had no effect on the potentiation induced by brief 5 Hz stimulation (Figure 1B and 1C). Therefore, it seems unlikely that β-adrenergic receptor activation is enhancing LTP by indirectly increasing NMDA receptor activity during 5 Hz stimulation, since this should enhance the modest LTP induced by brief 5 Hz stimulation trains. Instead, it seems to be down-regulating a process activated during longer periods of 5 Hz stimulation that inhibits the induction of LTP. What might this inhibitory process be? Previous studies have shown that 3 min of 5 Hz stimulation delivered shortly before or after high frequency stimulation inhibits LTP through a protein phosphatase-dependent mechanism (O'Dell and Kandel, 1994). Thus, although the protein kinases required for LTP are rapidly activated during 5 Hz stimulation, long trains of stimulation may also activate protein phosphatases that oppose the induction of LTP. Consistent with the notion that protein phosphatases are preferentially activated by longer trains of 5 Hz stimulation, we observed that while 3 min of 5 Hz stimulation delivered 2 min after high frequency stimulation inhibited high frequency stimulation–induced LTP, 30 s of 5 Hz stimulation had no effect (Figure 2A). Moreover, although it has previously been reported that high concentrations of norepinephrine (200 μM) enhance the reversal of LTP by 1 min of 5 Hz stimulation (Larson et al., 1993), we observed that selectively activating β-adrenergic receptors with 1.0 μM ISO completely prevented the inhibition of LTP by 3 min of 5 Hz stimulation (Figure 2B). There are numerous methodological differences between our experiments and those of Larson et al., and the reasons for the difference between our observations and theirs are not clear. However, because we observed that β-adrenergic receptor activation only enhances the ability of long trains of 5 Hz stimulation to induce LTP (see Figure 1), that long (but not short) trains of 5 Hz stimulation induce depotentiation, a depression of synaptic transmission at synapses that have undergone LTP (Figure 2A), and that ISO, like phosphatase inhibitors (O'Dell and Kandel, 1994), blocks depotentiation (Figure 2B), we investigated whether β-adrenergic receptor activation selectively enhances the induction of LTP during longer periods of 5 Hz stimulation by suppressing protein phosphatase activity, perhaps through a PKA-mediated phosphorylation of a regulatory protein, like inhibitor 1, that inhibits protein phosphatase 1 (Lisman, 1994).

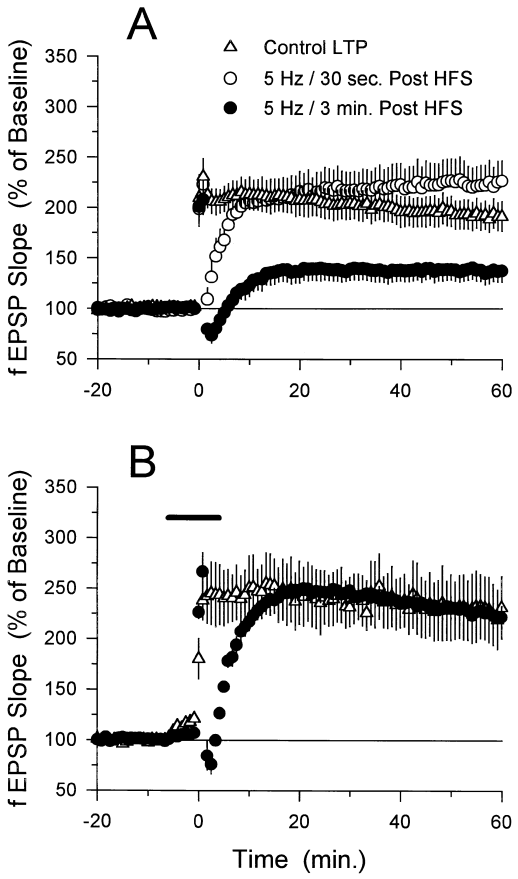

Figure 2. β-Adrenergic Receptor Activation Blocks Depotentiation Induced by Long Trains of 5 Hz Stimulation

(A) Three minutes, but not 30 s, of 5 Hz stimulation inhibits high frequency stimulation–induced LTP. LTP was induced with two trains of 100 Hz stimulation delivered at time = 0. In control experiments (triangles, n = 15), fEPSPs evoked 60 min after high frequency stimulation were 191.5% ± 14.7% of baseline. When 3 min of 5 Hz stimulation was delivered 2 min after high frequency stimulation (closed circles, n = 7), the amount of LTP measure 60 min after the high frequency stimulation was depressed (fEPSPs were 138.4% ± 11.2% of baseline, significantly different from control LTP, t(20) = 2.31, $p < 0.05$); 30 s of 5 Hz stimulation delivered 2 min after high frequency stimulation had no effect on LTP (open circles, n = 8, fEPSPs were 229.2% ± 22.8% of baseline, not significantly different from control LTP, t(21) = 1.45).

(B) ISO prevents the inhibition of LTP by 3 min of 5 Hz stimulation. In control experiments (triangles, n = 5), two trains of 100 Hz stimulation were delivered (at time = 0) in the presence of 1.0 μM ISO (indicated by the bar). fEPSPs were potentiated to 231.0% ± 36.0% of baseline 60 min after 100 Hz stimulation. When 3 min of 5 Hz stimulation was delivered after 100 Hz stimulation (all in the presence of 1.0 μM ISO, circles, n = 6), fEPSPs were 223.5% ± 12.05% of baseline (not significantly different from control LTP in ISO, t(4) = 0.2).

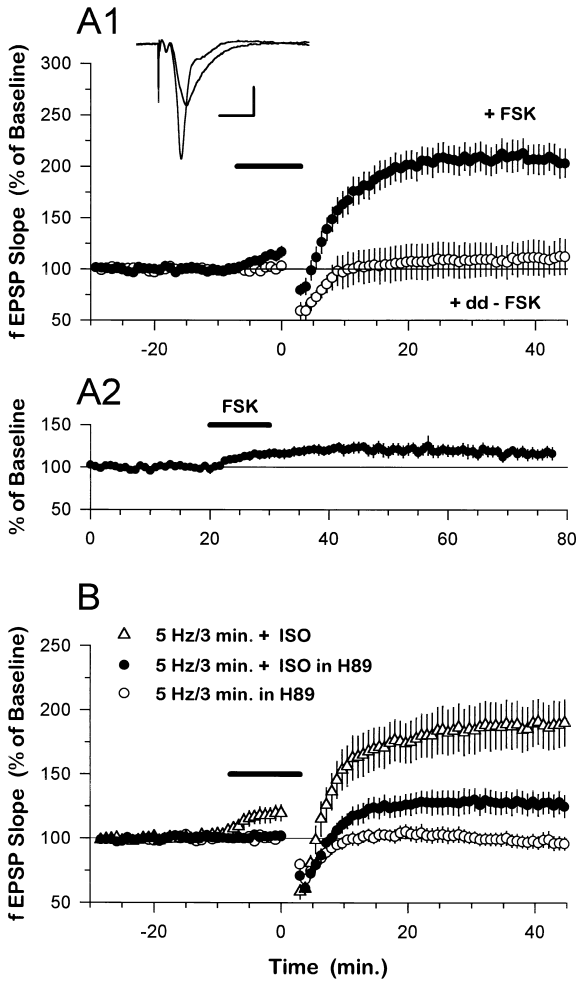

Figure 3. β-Adrenergic Receptor Activation Enhances the Induction of LTP by Long Trains of 5 Hz Stimulation through a PKA-Dependent Mechanism

(A1) fEPSPs were potentiated to 204.9% ± 14.3% of baseline when 5 Hz stimulation was delivered following a 10 min application (indicated by the bar) of 50 μM forskolin (FSK, 0.1% DMSO, closed symbols, n = 11, t(10) = 7.35, p < 0.01 compared with baseline). LTP was not induced by 5 Hz stimulation in the presence of 50 μM 1,9-dideoxy-forskolin (dd-FSK, 0.1% DMSO), an inactive FSK analog (open symbols, responses were 110.7% ± 18.2% of baseline, n = 6, t(5) = 0.54, not significant compared with baseline). The traces are fEPSPs recorded during baseline and 45 min after 5 Hz stimulation in FSK. Calibration bars are 2.0 mV and 5.0 ms.

(A2) Bath application of FSK alone produced a small enhancement of synaptic transmission that did not recover following FSK washout (fEPSPs were 115.4% ± 6.1% of baseline 45 min after a 10 min application of 50 μM FSK, n = 5, t(4) = 2.52, p < 0.05 compared with baseline).

(B) The protein kinase A inhibitor H89 inhibits LTP induced by 5 Hz stimulation in ISO. In vehicle control experiments (0.2% DMSO), fEPSPs were potentiated to 187.8% ± 18.7% of baseline 45 min after 3 min of 5 Hz stimulation in the presence of 0.5 μM ISO (open triangles, n = 9, ISO application indicated by the bar). The amount of potentiation induced by 5 Hz stimulation in ISO was significantly reduced in slices continuously incubated in 10 μM H89 (in 0.2% DMSO) for 1–4 hr prior to 5 Hz stimulation in ISO (closed circles, fEPSPs were 126.1% ± 8.5% of baseline, n = 9, t(8) = 3.01, p < 0.01 compared with ISO plus 5 Hz stimulation in the absence of H89); 3 min of 5 Hz stimulation alone had no lasting effect on synaptic transmission in H89-treated slices (open circles, fEPSPs were 98.6% ± 6.1% of baseline, n = 7, not significantly different from baseline, t(6) = 0.23).

This hypothesis suggests that β-adrenergic receptor activation enables the induction of LTP during 3 min of 5 Hz stimulation by stimulating cAMP production and activating PKA. Consistent with this, forskolin (50 μM), a direct activator of adenylyl cyclase, enabled the induction of LTP by 3 min of 5 Hz stimulation, while the inactive forskolin analog 1,9-dideoxy-forskolin had no effect (Figure 3A). Moreover, the potentiation produced by 3 min of 5 Hz stimulation in 0.5 μM ISO was significantly inhibited by H89 (10 μM, 1–4 hr pretreatment), a selective PKA inhibitor (Figure 3B). H89 had no effect on LTP induced with 100 Hz stimulation (in vehicle control experiments [0.2% DMSO], fEPSPs were 183.0% ± 27.4% of baseline 60 min posttetanus, n = 7, and were 183.0% ± 21.1% of baseline in slices treated with 10 μM H89, n = 8), suggesting that PKA is not an essential component of the protein kinase cascade responsible for the early phases of LTP (Weisskopf et al., 1994; however, see Blitzer et al., 1995; Musgrave et al., 1993). Instead, in addition to its role in the late, protein synthesis–dependent phase of LTP (Frey et al., 1993), the present data show that PKA may also be part of a modulatory pathway that enables the induction of LTP by low frequency stimulation. In addition, since H89 had no effect on LTP induced with high frequency stimulation, it seems unlikely that H89 inhibited the induction of LTP by 5 Hz stimulation in ISO through nonselective effects on the serine/threonine and tyrosine kinases required for the induction of LTP by high frequency stimulation (Bliss and Collingridge, 1993).

Does β-adrenergic receptor activation selectively enable the induction of LTP by long trains of 5 Hz stimulation through a PKA-mediated inhibition of protein phosphatases? To address this question, we examined whether the protein phosphatase 1 and 2A inhibitor calyculin A (CA, Ishihara et al., 1989) mimicked the effects of ISO on the induction of LTP by 5 Hz stimulation. As shown in Figures 4A–4C, in slices pretreated with CA (0.5–1.0 μM), both 1 and 3 min trains of 5 Hz stimulation–induced LTP (fEPSPs were potentiated to 171.8% ± 15.2%, n = 11, and 148.1% ± 10.4% of baseline, n = 14, for 1 and 3 min 5 Hz trains, respectively), while there was no effect on the potentiation induced by either 15 or 30 s of 5 Hz stimulation. Importantly, repetitive 1 Hz simulation in the presence of CA can enhance synaptic transmission in the CA1 region of the hippocampus through changes in presynaptic fiber excitability (Herron and Malenka, 1994). However, this does not seem to contribute to the induction of LTP by 5 Hz stimulation in the presence of CA, since presynaptic excitability changes following 1 Hz stimulation in CA are not blocked by glutamate receptor antagonists (Herron and Malenka, 1994), but we found that APV (100 μM) inhibited the potentiation induced by 1 min of 5 Hz stimulation in CA-treated slices (fEPSPs were 108.9% ± 5.83% of baseline, n = 6, t(5) = 3.87, p < 0.01 compared with 5 Hz for 1 min stimulation in the absence of APV).

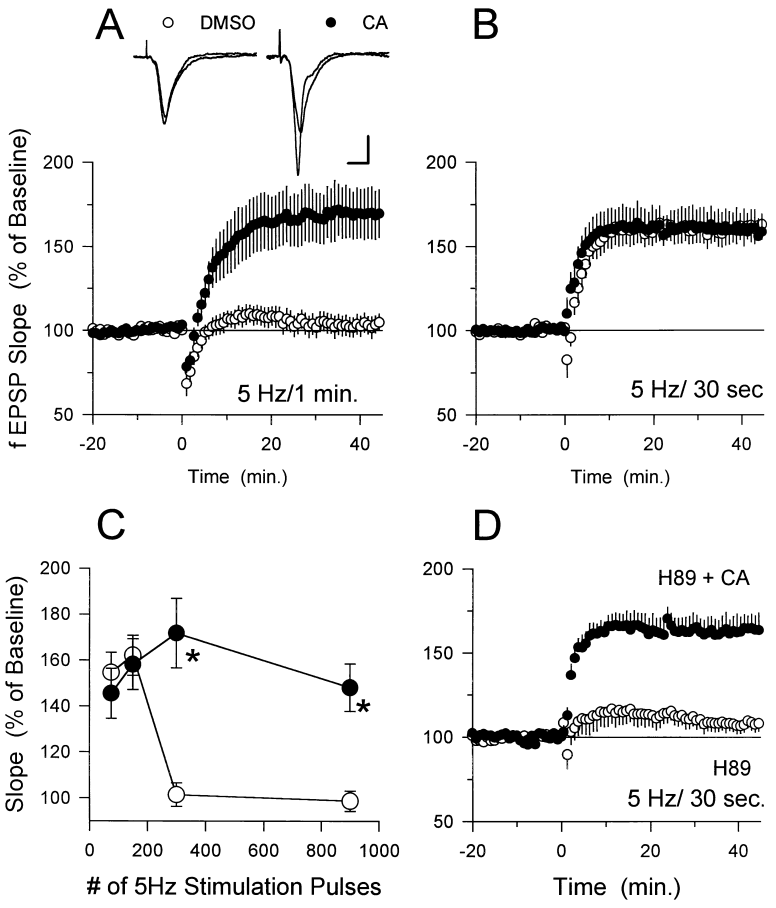

Figure 4. The Protein Phosphatase 1 and 2A Inhibitor CA Selectively Enhances the Induction of LTP by Long Trains of 5 Hz Stimulation

(A) In vehicle control experiments (0.2% DMSO, open symbols), LTP was not induced by 1 min of 5 Hz stimulation (fEPSPs were 109.5% ± 15.1% of baseline 45 min after 5 Hz stimulation, n = 7, t(6) = 0.30, not significantly different from baseline). In slices preincubated in 0.5–1.0 μM CA for at least 45 min, fEPSPs were potentiated to 171.8% ± 15.2% of baseline following 1 min of 5 Hz stimulation (closed symbols, n = 11, t(6) = 4.39, $p < 0.01$ compared with 5 Hz for 1 min control experiments). The β-blocker timolol (10 μM) had no effect on the potentiation induced by 5 Hz for 1 min stimulation in CA-treated slices (fEPSPs were 175.5% ± 19.1% of baseline, n = 4, data not shown). The traces are fEPSPs recorded during baseline and 45 min after 5 Hz stimulation in a control experiment (0.2% DMSO) and in a CA-treated slice. Calibration bars are 2.0 mV and 5.0 ms.

(B) The potentiation induced by 30 s of 5 Hz stimulation in control experiments (0.2% DMSO, open symbols) is not enhanced by preincubation in CA (closed symbols). In control experiments, fEPSPs were 162.1% ± 8.7% of baseline (n = 8) 45 min after 30 s of 5 Hz stimulation and were 158.2% ± 10.9% of baseline in CA-treated slices (n = 8, not significantly different, t(14) = 0.28).

(C) Summary of the effects of 0.5–1.0 μM CA on changes in synaptic strength induced by different amounts of 5 Hz stimulation. CA had no effect on the amount of LTP induced by short trains of 5 Hz stimulation (15 and 30 s), but significantly enhanced (asterisk, $p < 0.01$) the potentiation induce by longer trains of 5 Hz stimulation (1 or 3 min).

(D) Pretreatment (1–4 hr) with the PKA inhibitor H89 (10 μM) blocked the induction of LTP by 30 s of 5 Hz stimulation (open symbols, fEPSPs were 108.4% ± 4.7% of baseline 45 min after 5 Hz stimulation, n = 8, significantly different from 5 Hz for 30 s controls (see [B]), t(14) = 5.42, $p < 0.01$). Pretreatment (at least 45 min) with the phosphatase inhibitor CA (0.75 μM) restored the ability of 30 s of 5 Hz stimulation to induce LTP in H89-treated slices (closed symbols, fEPSPs were 165.6% ± 8.41% of baseline, n = 6).

Studies of hippocampal LTD suggest that low frequency synaptic stimulation activates protein phosphatase 1 via activation of the Ca^{2+}-dependent phosphatase calcineurin and subsequent dephosphorylation of a phosphatase 1 regulatory protein such as inhibitor 1 (Mulkey et al., 1993, 1994). It was thus somewhat surprising that β-adrenergic receptor activation or pharmacological inhibition of protein phosphatase 1 had no effect on LTP induced by short trains of 5 Hz stimulation, since even short periods of 5 Hz stimulation could at least partially activate protein phosphatases and inhibiting these phosphatases should favor the induction of LTP. One possibility is that the levels, or more likely the duration, of Ca^{2+} influx during short trains of 5 Hz stimulation is simply not sufficient to activate the calcineurin-dependent pathway leading to protein phosphatase 1 activation. Alternatively, since NMDA-receptor mediated increases in intracellular Ca^{2+} will also increase intracellular levels of cAMP (Chetkovich and Sweatt, 1993) through stimulation of Ca^{2+}-dependent isoforms of adenylyl cyclase (Blitzer et al., 1995; Cooper et al., 1995), a second possibility is that, while calcineurin may be activated during short trains of 5 Hz stimulation, PKA activation prevents protein phosphatase 1 activation by opposing the dephosphorylation of inhibitor 1 by calcineurin. This second hypothesis suggests that PKA inhibitors should block the induction of LTP by short trains of 5 Hz stimulation and, if PKA activation normally acts by suppressing the activation of protein phosphatase 1, then the ability of PKA inhibitors to block LTP should be overcome by pharmacological inhibition of protein phosphatase 1. Consistent with this, we observed that the induction of LTP by 30 s of 5 Hz stimulation was blocked by the PKA inhibitor H89 and that this inhibition was overcome by preincubation in CA (Figure 4D).

Discussion

β-Adrenergic Receptor Activation Modulates the Induction of CA1 LTP, Perhaps by Opposing Protein Phosphatase Activity

Our results show that β-adrenergic receptor activation strongly modulates the induction of LTP in the CA1 region of the hippocampus. However, this modulation is highly activity-dependent, β-adrenergic receptor activation had no effect on the amount of potentiation induced

by short periods of 5 Hz stimulation, but was required for the induction of LTP by long trains of 5 Hz stimulation. How is this selective, activity-dependent modulation of LTP achieved? Our results, along with previous findings (O'Dell and Kandel, 1994), suggest that long trains of 5 Hz stimulation fail to induce LTP not because they fail to activate the protein kinases responsible for LTP but because they also activate protein phosphatases that oppose the induction of LTP. Recent reports suggest that for certain patterns of synaptic stimulation PKA may have an important role in CA1 LTP by activating a gate-like signaling pathway that enables the induction of LTP by inhibiting protein phosphatases (Blitzer et al., 1995; Iyengar, 1996). Although our results do not conclusively demonstrate the precise mechanism by which β-adrenergic receptor activation modulates low frequency stimulation-induced LTP, they are consistent with the hypothesis that β-adrenergic receptor activation acts through this PKA-dependent gate-like mechanism and thus enables the induction of LTP by patterns of synaptic activity that activate protein phosphatases, while having little effect on the induction of LTP by patterns of synaptic activity that do not significantly activate protein phosphatases. Importantly, we have not tested whether β-adrenergic receptor activation and protein phosphatases specifically interact postsynaptically to control the induction of LTP by 5 Hz stimulation. Thus, it remains a possibility that β-adrenergic receptor–mediated PKA activation and protein phosphatases interact presynaptically, or even act through signaling pathways on opposite sides of the synapse, to modulate the induction of LTP. However, our hypothesis that the activity-dependent effects of β-adrenergic receptor activation on low frequency stimulation–induced LTP arise via PKA modulation of protein phosphatases is consistent with recent reports showing that postsynaptic PKA modulation of postsynaptic protein phosphatases has an important role in the induction of high frequency stimulation–induced LTP (Blitzer et al., 1995) and that ISO, by activating PKA, antagonizes the effects of calcineurin on the NMDA receptor ion channel in cultured hippocampal neurons (Raman et al., 1996).

The mechanisms whereby protein phosphatases are selectively activated during long, but not short, trains of 5 Hz stimulation are unclear. Our results shown in Figure 4D are consistent with the hypothesis that PKA activation following increases in cAMP generated by Ca^{2+}-sensitive isoforms of adenylyl cyclase opposes the activation of protein phosphatase 1 by calcineurin during short trains of 5 Hz stimulation. However, it appears that this mechanism is unable to prevent protein phosphatase 1 activation during longer trains of 5 Hz stimulation. One reason for this may be that during longer trains of 5 Hz stimulation Ca^{2+} influx through the NMDA receptor ion channels activates calcineurin, which in turn inhibits NMDA receptor ion channel activity (Lieberman and Mody, 1994; Tong et al., 1995). As a result of this inhibitory feedback modulation of the NMDA receptor ion channel, intracellular Ca^{2+} levels may fall below that necessary to stimulate Ca^{2+}-sensitive adenylyl cyclases and activate PKA. Unopposed by PKA, calcineurin would now be able to inhibit the induction of LTP by activating protein phosphatase 1 through dephosphorylation of inhibitor 1. Importantly, this model, and our interpretation of how β-adrenergic receptor activation modulates CA1 LTP, assumes a pivotal role for inhibitor 1 as the site where PKA and calcineurin converge to control phosphatase activity and thus modulate the induction of LTP. However, it has recently been demonstrated that β-adrenergic receptor activation, through activation of PKA, can directly overcome the calcineurin-mediated inhibition of NMDA receptor ion channel activity (Raman et al., 1996). Thus, β-adrenergic receptor activation may enable the induction of LTP by long trains of 5 Hz stimulation by opposing the actions of calcineurin on NMDA receptor–mediated Ca^{2+} influx instead of (or perhaps in addition to) opposing the actions of calcineurin on inhibitor 1.

β-Adrenergic Receptor Modulation of CA1 LTP May Be Important in Memory Formation

Although repetitive activation of excitatory synapses in the CA1 region of the hippocampus at 5 Hz is sufficient to activate the protein kinase–dependent signaling cascade responsible for LTP, our results suggest that prolonged periods of synaptic activity at this frequency will also activate protein phosphatases that inhibit the induction of LTP. This may provide a mechanism for preventing persistent changes in synaptic strength by low frequency (5 Hz) patterns of synaptic activity that occur for extended periods of time in the absence of behaviorally significant events. However, our results suggest that activation of noradrenergic projections to the hippocampus (Loy et al., 1980), for instance during periods of heightened emotional arousal, will strongly enhance the induction of LTP by these patterns of synaptic activity and suggest a cellular mechanism for understanding the role of β-adrenergic receptor activation in the enhancement of memory formation that occurs during emotional experiences (McGaugh, 1989, 1990; Cahill et al., 1994; also see Dahl and Li, 1994; Huang and Kandel, 1996). Moreover, G_i-linked receptors for serotonin, GABA, and glutamate can potentiate increases in cAMP following β-adrenergic receptor activation (Andrade, 1993; Gereau and Conn, 1994b; Bourne and Nicoll, 1993) presumably because G_i βγ subunits enhance the activity of the type II adenylyl cyclase stimulated following β-adrenergic receptor activation (Tang and Gilman, 1991; Federman et al., 1992; Cooper et al., 1995). This suggests the intriguing possibility that the signaling processes activated by β-receptor activation not only have a powerful modulatory influence on the induction of LTP but may in turn be modulated by other transmitters. This could provide an important point of convergence where the influence of numerous neurotransmitters could be integrated to control the induction of LTP. If so, this adds an extraordinary level of flexibility and complexity to how synapses may use a LTP-like mechanism to persistently alter the strength of excitatory synaptic transmission and thus form new memories.

Experimental Procedures

Standard techniques (Thomas and O'Dell, 1995) were used to record fEPSPs in stratum radiatum of the CA1 region of 400 μm thick slices of C57BL/6 mouse hippocampus. Slices were maintained (at

30°C–31°C) in an interface type recording chamber perfused (1–3 ml/min) with a mouse artificial cerebrospinal fluid (ACSF) consisting of 124 mM NaCl, 4.4 mM KCl, 25 mM Na_2HCO_3, 1.0 mM Na_2PO_4, 1.2 mM $MgSO_4$, 2.0 mM $CaCl_2$, and 10 mM glucose, gassed with 95% O_2, 5% CO_2. In all experiments, fEPSPs were evoked (at 0.02 Hz) using stimulation strengths sufficient to elicit fEPSPs that were approximately 50% of the maximal fEPSP amplitude. Using these conditions, we did not observe robust LTD of basal synaptic transmission (fEPSPs recorded 45 min after 15 min of 1 Hz stimulation were 93.2% ± 4.2% of baseline, n = 8, t(7) = 1.60, not significant compared with baseline) in slices from the 3- to 5-week-old animals used in these experiments. High frequency stimulation–induced LTP was elicited by two 1 s long trains of 100 Hz stimulation (intertrain interval = 10 s). In general, experiments were interleaved such that slices from the same animals were used in both control experiments and those testing the effects of various drugs. In occlusion experiments, saturating levels of LTP were induced with three trains of 100 Hz stimulation (1.0 s duration, intertrain interval = 10 s); 20 min after the last high frequency train, the stimulation intensity was reduced and a new 20 min baseline was recorded prior to any further experimental manipulations. In control experiments, two trains of 100 Hz stimulation delivered after the second baseline period failed to elicit additional LTP (fEPSPs 60 min after the two 100 Hz trains were 108.2% ± 6.31% of the new baseline, n = 6, t(5) = 1.07, not significantly different from the second baseline level), indicating that the synaptic mechanisms for LTP had been saturated. Results are reported as mean ± SEM. Statistical comparisons (paired and unpaired Student t tests) were performed using responses measured 45 min after 5 Hz stimulation or 60 min after 100 Hz stimulation.

All drugs were bath applied following dilution into ACSF from concentrated stock solutions. APV (±2-amino-5-phosphonopentanoic acid), ISO (R(−)-isoproterenol (+)-bitartrate), and timolol were purchased from Research Biochemical International (RBI). FSK (forskolin) and dd-FSK (1,9 dideoxy-forskolin) were purchased from RBI and prepared as concentrated stock solutions (50 mM) in dimethyl sulfoxide (DMSO). Calyculin A (LC Laboratories) and H89 (Biomol) were prepared as concentrated stock solutions in DMSO (1 mM and 5 mM, respectively) and stored at −20°C (typically no more than 4 days prior to use). For experiments with H89, slices were continuously bathed in H89 containing ACSF for 1–4 hr prior to the experiment and H89 was present throughout the experiment (see Weisskopf et al., 1994). CA was applied for 45–60 min prior to an experiment. The slices were then switched to normal ACSF and 5 Hz stimulation was delivered within 30 min following washout of CA from the recording chamber.

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