Postsynaptic Kinase Signaling Underlies Inhibitory Synaptic Plasticity in the Lateral Superior Olive

Vibhakar C. Kotak,[1] Dan H. Sanes[1,2]

[1] Center for Neural Science, 4 Washington Place, New York University, New York, New York 10003

[2] Department of Biology, New York University, New York, New York 10003

Received 22 February 2002; accepted 7 June 2002

ABSTRACT: In the auditory system, inhibitory transmission from the medial nucleus of the trapezoid body (MNTB) to neurons of the lateral superior olivary nucleus (LSO) undergoes activity-dependent long-term depression, and may be associated with developmental elimination of these synapses [Sanes DH, Friauf E (2000). Review: development and influence of inhibition in the laterial superior olivary nucleus. Hear Res 147: 46–58]. Although $GABA_B$ receptor activation and postsynaptic free calcium are implicated in this depression, little is known about intracellular signaling mechanisms in this or other forms of inhibitory plasticity. In this study, we asked whether the calcium dependency of inhibitory depression was associated with the activation of calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), and/or cAMP-dependent protein kinase A (PKA). Whole-cell voltage-clamp recordings were obtained from LSO neurons in a brain slice preparation, permitting for the selective pharmacologic manipulation of individual postsynaptic LSO neurons. Inclusion of a CaMKII antagonist (KN-62) in the internal pipet solution blocked inhibitory synaptic depression. A second CaMKII inhibitor (autocamtide peptide fragment) significantly decreased inhibitory depression. Inclusion of a specific antagonist of protein kinase C (PKC fragment 19–36) in the internal recording solution also blocked inhibitory depression. To test involvement of a cAMP-dependent intracellular cascade, two different manipulations were performed. Inclusion of PKA antagonists (Rp-cAMPS *or* a cAMP dependent protein kinase inhibitor peptide) prevented inhibitory depression. In contrast, when a nonhydrolyzable cAMP analog (Sp-cAMPS) was permitted to enter the postsynaptic cell, the MNTB-evoked IPSCs became depressed in the absence of low-frequency stimulation. Thus, three key postsynaptic kinases, CaMKII, PKC, and PKA, participate in the activity-dependent depression of inhibitory MNTB-LSO synapses during postnatal development. J Neurobiol 53: 36–43, 2002

Keywords: **inhibition; long-term depression; development; CaMKII; PKC; PKA; LSO; MNTB; auditory**

INTRODUCTION

Modification of synaptic strength occurs at developing and mature synapses. During development, this plasticity may contribute to the addition or loss of synaptic connections, while in adult animals it is associated with environmentally induced perceptual changes or pathophysiologic conditions. In adult models of excitatory synaptic plasticity, glutamatergic synapses in the hippocampus and cerebellum display long-term potentiation or depression (Bliss and Lømo, 1973; Madison et al., 1991; Mulkey and Malenka, 1992; Linden and Connor, 1995). Furthermore, CaMKII, PKC, and PKA have been implicated in the adjustment of excitatory synaptic gain. For example, LTD induction at naive glutamatergic synapses is accompanied by dephosphorylation of a PKA-sensitive site on the AMPA receptor. At potentiated syn-

Correspondence to: D.H. Sanes (sanes@cns.nyu.edu).
Contract grant sponsor: NIH; contract grant number: DC00540.

DOI 10.1002/neu.10107

apses, the CaMKII site becomes dephosphorylated. In contrast, LTP induction at naive or depressed synapses is associated with phosphorylation of the CaMKII and PKA sites, respectively (Lee et al., 1998, 2000). In developmental models, such processes may be linked to the anatomical reconfiguration of synapses, establishing the appropriate number or type of connections (Sanes et al., 2000). In fact, CaMKII, PKC, and PKA have each been implicated in developmental synaptic plasticity (Wang et al., 1994; Kano et al., 1995; Zou and Cline, 1996; Davis et al., 1996; Sun and Schacher, 1996; Boulanger and Poo, 1999).

Whereas there are several reports of inhibitory synaptic plasticity, few of the biochemical mechanisms have been explored. Such models include GABAergic LTP and LTD in the hippocampus (Morishita and Sastry, 1991), glycinergic LTP at goldfish Mauthner neurons (Oda et al., 1995, 1998), GABAergic LTP in the visual cortex (Komatsu, 1994, 1996; Komatsu and Yoshimura, 2000), rebound potentiation in cerebellar Purkinje cells (Kano et al., 1992), an age-dependent LTD in the developing LSO (Kotak and Sanes, 2000; Kotak et al., 2001), and disuse-dependent inhibitory depression in the inferior colliculus (Vale and Sanes, 2000). Chloride homeostatic mechanisms appear to play a critical role in regulating inhibitory strength (Kakazu et al., 1999; Vale and Sanes, 2000; Ganguly et al., 2001), but do not appear to underlay the inhibitory LTD in our system (Kotak and Sanes, 2000).

It has been previously reported that inhibitory synapses in the developing superior olive display use-dependent depression during the period of maturation when inhibitory synapses undergo rearrangement (Sanes and Takács, 1993; Kotak and Sanes, 2000; Kapfer et al., 2002). Low-frequency stimulation (LFS) of inhibitory afferents from the MNTB leads to a robust depression recorded at the postsynaptic LSO neuron, and this phenomenon is age dependent (Kotak and Sanes, 2000).

The present study asks whether inhibitory LTD employs intracellular signaling mechanisms known to operate at excitatory synapses. Because postsynaptic free calcium increases the magnitude of inhibitory depression in the LSO (Kotak and Sanes, 2000), we initially asked whether CaMKII mediated the phenomenon. We then explored whether PKC and PKA also participated in inhibitory depression. Using specific antagonists and agonists, restricted selectively to the recorded postsynaptic neuron, we provide data that support involvement of all three enzymes in inhibitory LTD.

METHODS

Gerbil pups *(Meriones unguiculatus)* aged postnatal (P) days 7–12 were used to generate 300 μM transverse brain slices containing the MNTB-LSO circuitry. The artificial cerebrospinal fluid (ACSF) contained (in m*M*): 125 NaCl, 4 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 24 $NaHCO_3$, 15 glucose, 2.4 $CaCl_2$, and 0.4 L-ascorbic acid (pH = 7.3 when bubbled with 95% O_2/5% CO_2). ACSF was continuously superfused in the recording chamber at 3–4 mL per min at room temperature (22–24°C). Whole-cell voltage-clamp recordings were obtained from LSO neurons (Warner PC-501A), and 200-μs electrical pulses were delivered directly to the MNTB (Kotak et al., 1998). Recording electrodes were fabricated from borosylicate glass microcapillaries (1.5 mm o.d.), and when filled with internal solution, their resistance ranged 5–10 MΩ. The internal patch solution contained (in m*M*) 127.5 cesium gluconate, 0.6 EGTA, 10 HEPES, 2 $MgCl_2$, 5 KCl, 2 ATP, 0.3 GTP, and QX-314 (pH = 7.2). Access resistance was balanced throughout the recordings and ranged between 10 and 40 MΩ.

Induction of long-lasting inhibitory depression is described in a previous report (Kotak and Sanes, 2000). Briefly, MNTB-evoked maximum amplitude inhibitory postsynaptic currents (IPSCs) were first acquired during a 15-min control period at a holding potential of 0 mV. The MNTB was then activated with low frequency stimulation (LFS: 1 Hz for 15 min). Thereafter, MNTB-evoked IPSCs were monitored for an hour. In all experiments, ionotropic glutamatergic activity was blocked by 4 m*M* kynurenic acid in the ACSF (pH 7.3 after bubbling with O_2/CO_2).

To selectively manipulate the postysnaptic cell's internal environment and disrupt specific intracellular biochemical pathways, several pharmacologic agents were included in the internal recording solution, each in a separate set of experiments. Manipulated neurons were exposed to one specific drug when the membrane patch was ruptured. Control recordings were performed using normal internal recording solution without any added agent, as reported previously (Kotak and Sanes, 2000). In either case, a 4-min waiting period was observed following membrane rupture and before the first IPSC recording. During this time, sodium action potentials were blocked by diffusion of QX-314 from the internal solution. Therefore, we assume that the low molecular weight pharmacologic agents also infiltrated the recorded neuron in this time period. To block CaMKII in the postsynaptic LSO neuron, either KN-62 (5–15 μM) *or* autocamtide peptide fragment (0.75–1.5 μM) was included in the internal pipet solution. The activity of PKC was blocked with 0.5–2 μM Protein Kinase C fragment 19–36 (Sigma). To block PKA, a competitive antagonist of cAMP, Rp-cAMPS (10 μM) was added to the recording solution. A second protein kinase inhibitor peptide (Sigma) that binds to the catalytic subunit of cAMP dependent protein kinase (5–10 μM PK inhibitor peptide) was used in a separate set of recordings. The effect of activating PKA with a cAMP analog (0.5–2 μM Sp-cAMPS) on evoked IPSCs was examined in the absence of LFS for about an hour. For these recordings, IPSCs were acquired every 10 min so that

alterations in IPSC amplitude could be examined with minimal afferent activity. For example, some depression in IPSCs is seen even in the absence of LFS (Kotak and Sanes, 2000). For this manipulation we obtained an additional set of control recordings for about an hour with stimuli delivered at identical time intervals. For all drug treatments, there remains a possibility of intracellular alterations before the first IPSC is acquired.

The data were collected using a Macintosh PPC running a custom IGOR (WaveMetrics, v3.14) macro, and they were analyzed off-line using a second IGOR macro (Kotak et al., 2001). The results are presented as mean and standard error of the mean. Comparisons were performed with an ANOVA followed by paired comparisons using the Student's *t* tests (JMP statistical software).

RESULTS

The data presented below describe whole-cell voltage-clamp recordings from 56 LSO neurons. Each recording was obtained from a fresh brain slice preparation. Control recordings were obtained under identical experimental conditions in slices from age-matched gerbils ($N = 7$). For control experiments in which LTD was induced by low-frequency stimulation of MNTB (Figs. 1–3), the magnitude of LTD was nearly identical (~65% depression) to previously published data from 21 LSO neurons (~60% depression; Kotak and Sanes, 2000).

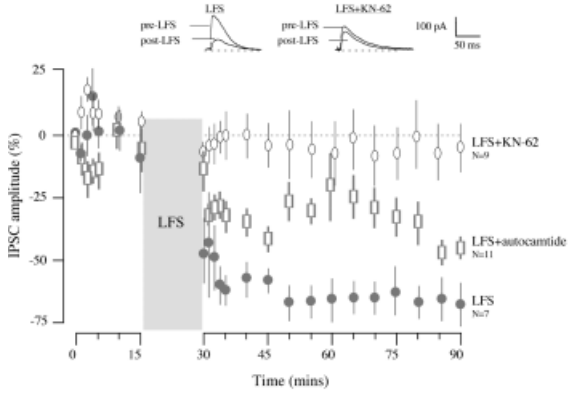

Figure 1 Inhibitory depression is CaMKII dependent. The graph compares LTD in P7-12 neurons that were recorded with normal pipet solution (solid circles) with data from recordings with 5–15 μM KN-62 (open circles) or autocamtide peptide (open rectangles) in the pipet solution (CaMKII antagonists). The percent reduction in IPSC amplitude (±SEM) shows that inhibitory depression was abolished by KN-62. Representative MNTB-evoked IPSCs before and after LFS are shown for recordings with control and KN-62–containing pipet solution (top). Inclusion of a different CAMKII blocker (autocamtide peptide fragment) also reduced inhibitory depression. See Results for statistical comparisons.

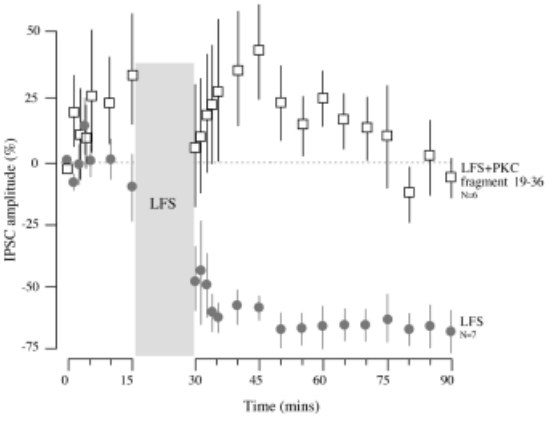

Figure 2 Inhibitory depression is protein kinase C (PKC) dependent. The graph compares inhibitory LTD in P7-12 neurons that were recorded with normal pipet solution (filled circles), and pipet solution that contained a specific inhibitor of PKC, the PKC fragment 19–36 (500 nM–2 μM, open squares), which blocked the depression. See Results for statistical comparisons.

Because a postsynaptic calcium chelator eliminates inhibitory depression (Kotak and Sanes, 2000), we first asked whether CaMKII was involved. In separate sets of recordings, two different antagonists of CaMKII (KN-62, 5–15 μM; autocamtide peptide fragment; 0.75–1.5 μM) were included in the internal pipet solution. As shown in Figure 1, both manipulations suppressed the inhibitory LTD produced by LFS. The mean percent change in IPSC amplitude within a group of neurons was calculated by comparing the average amplitudes of three IPSCs recorded at 50–60 min post-LFS with the initial pre-LFS maximum amplitude IPSC (Student's *t* test: Control: $t = 10.9$, $df = 13$, $p < .0001$, $n = 7$; KN-62-treated: $t = 1.1$, $df = 17$, $p > .2$, $n = 9$; autocamtide-treated: $t = 3.5$, $df = 21$, $p < .002$, $n = 11$). The efficacy of the two CaMKII inhibitors was not the same: KN-62 blocked depression completely, while the autocamtide peptide was less effective (Fig. 1). To determine whether inhibitory LTD was suppressed in autocamtide-treated neuorns, we compared the post-LFS IPSCs amplitudes of autocamtide-treated neurons to controls ($t = 4.04$, $df = 17$, $p = .009$, $n = 18$). Thus, the autocamtide peptide-treated IPSCs were significantly less depressed than control IPSCs.

In a separate set of recordings, a specific blocker of PKC was included in the internal recording solution (0.5–2 μM Protein Kinase C fragment 19–36). As shown in Figure 2, this manipulation also disrupted inhibitory depression. The mean percent change in

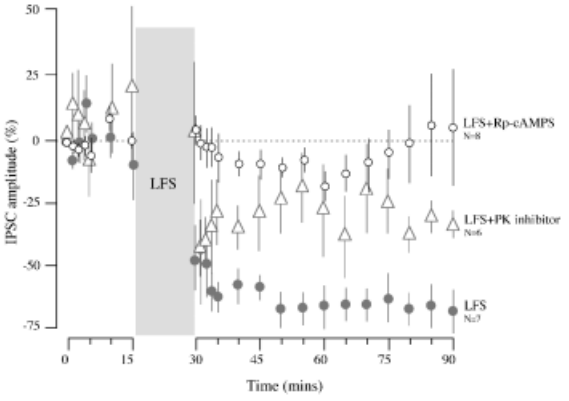

Figure 3 Inhibitory depression is cAMP dependent. The graph compares inhibitory LTD in P7-12 neurons that were recorded with normal pipet solution (filled circles) and pipet solution that contained Rp-cAMPS (10 μM, open circles) or a cyclic AMP-dependent protein kinase inhibitor (5–10 μM) (PKA antagonists, open triangles, SEMs are displayed as +, −, or in both directions for graphic clarity), which blocked the depression. See Results for statistical comparisons.

IPSCs was calculated by comparing the average amplitudes of three IPSCs recorded at 50–60 min post-LFS with the initial pre-LFS maximum amplitude IPSC (Control, same as above; PKC fragment 19–36-treated: $t = -0.57$, $df = 11$, $p = .57$, $n = 6$). Therefore, blockade of postsynaptic PKC signaling completely eliminated inhibitory LTD.

In a third set of experiments, the involvement of cAMP-dependent signalling was tested. Two different antagonists of PKA were employed. First, inclusion of the PKA inhibitor, 10 μM Rp-cAMPS, in the internal pipet solution was shown to block activity-dependent inhibitory LTD (Fig. 3). The mean percent change in IPSCs was calculated by comparing the average amplitudes of three IPSCs recorded at 50–60 min post-LFS with the initial pre-LFS maximum amplitude IPSC (Control, same as above; Rp-cAMPS-treated: $t = -0.02$, $df = 15$, $p = .9$, $n = 8$). A second PKA antagonist (5–10 μM protein kinase inhibitor peptide) that binds to the catalytic subunit of cAMP-dependent protein kinase reduced, but did not eliminate, inhibitory LTD (post-LFS vs. pre-LFS: $t = 6$, $df = 9$, $p = .005$, $n = 5$). A comparison of protein kinase inhibitor peptide-treated post-LFS IPSCs with those from controls demonstrated that this PKA antagonist reduced inhibitory LTD significantly ($t = 3.9$, $df = 11$, $p = 0.002$, $n = 12$).

Inclusion of a cAMP analog (0.5–2 μM Sp-cAMPS) in the recording solution induced a gradual and significant reduction in the MNTB-evoked IPSCs in the *absence* of low-frequency stimulation of the MNTB (Fig. 4). Statistical comparisons were made between the initial IPSCs and IPSCs at 10, 30, or 50 min for both control and Sp-cAMPS–treated neurons (ANOVA: $F = 4.9$, $df = 5$, $p = .003$; paired comparisons of initial IPSCs vs. IPSCs at 10 min for Sp-cAMPS–treated neurons: $t = 3.6$, $df = 7$, $p = .01$; initial IPSCs vs. IPSCs at 30 min for Sp-cAMPS–treated neurons: t=3.8, df=7, p=0.008; initial IPSCs vs IPSCs at 50 min for Sp-cAMPS-treated neurons: $t = 5.9$, $df = 7$, $p = .001$; statistical differences between initial IPSCs and IPSCs at 20 min and 40 min are not shown). There was no significant difference in IPSC size for control neurons at identical recording time intervals, although some facilitation was observed (ANOVA, $F = 1.99$, $p > .1$, $n = 6$).

DISCUSSION

We have previously shown that LTD of inhibitory transmission at the developing LSO is greatest before hearing onset, and declines by postnatal days 17–19 (Kotak and Sanes, 2000). Inhibitory synapse elimination in both the LSO and medial superior olivary nucleus (MSO) occurs during this time period, and depends on an intact cochlea (Sanes and Siverls, 1991; Sanes and Takács, 1993; Kapfer et al., 2002), leading us to hypothesize that inhibitory LTD may contribute to the elimination of these synapses.

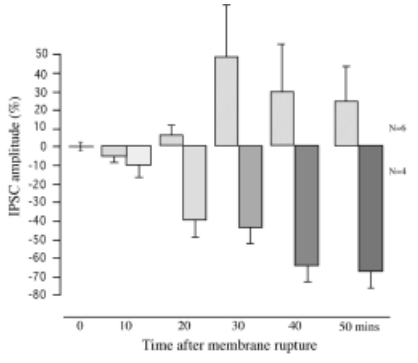

Figure 4 Inclusion of a cAMP analog (Sp-cAMPS, 0.5 μM–2 μM) depresses inhibition without LFS. IPSCs were recorded every 10 min with normal internal recording solution, and in separate experiments with internal recording solution added with Sp-cAMPS. Each bar is a mean of 3 IPSCs (±S.E.M.). For control recordings (hatched bars), an apparent facilitation of IPSCs was noticed, but there was no statistical difference between initial IPSCs and IPSCs at 10, 20, 30, 40, or 50 min (see Results). In contrast, for Sp-cAMPS recordings (gray/filled bars), IPSC amplitudes decreased significantly during first 10 min when compared to initial IPSCs, and continued to depress significantly thereafter. See Results for statistical comparisons.

In a recent study, we showed that $GABA_B$ receptor function is necessary for the expression of inhibitory LTD, and Trk receptor activity can also depress inhibitory synaptic transmission (Kotak et al., 2001). Therefore, the present study was conducted to determine the intracellular signaling mechanisms specifically operative at the postsynaptic site that lead to synaptic depression. Because it is possible to selectively block or activate enzymes in a single postsynaptic neuron by placing pharmacologic agents in the internal recording solution, our strategy was to focus exclusively on postsynaptic signaling mechanisms. Our data strongly suggest that three postsynaptic signals, CaMKII, PKC, and PKA, are involved in activity-dependent inhibitory LTD. However, it remains to be determined whether the $GABA_B$ or Trk receptors act via any of these signaling pathways. It is unlikely that inhibitory synaptic depression is due to a nonphysiologic alteration because the effect is observed with both current- and voltage-clamp recordings solutions, depression is blocked with several different pharmacological manipulations of the postsynaptic neuron (including those presented here), and a selective $GABA_B$ receptor antagonist blocks depression (Kotak and Sanes, 2000; Kotak et al., 2001).

The chelation of postsynaptic calcium prevents inhibitory depression (Kotak and Sanes, 2000), and we first asked whether this form of plasticity was dependent on activation of CaMKII or PKC, because both these enzymes are known to be modulated by intracellular calcium. Our data indicate that increased activity of both CaMKII or PKC are linked to inhibitory LTD. Pharmacologic blockade of CaMKII in the postsynaptic LSO neuron reduces depression (Fig. 1) while inclusion of the PKC fragment in the recording solution abolished depression (Fig. 2). For CamKII, the greater effect of KN-62 compared to autocamtide (Fig. 1) suggests a broader action of KN-62. For example, KN-62 has been shown to block calcium channels (Shira and Pearson, 1995).

Studies on synaptogenesis suggest that CaMKII does play a role in determining the number of synaptic contacts during development. In transgenic *Xenopus* tadpoles that express a constitutively active form of CaMKII in postsynaptic tectal neurons *in vivo*, the retinal ganglion cell arborizations are much simpler than they are in controls animals, suggesting that fewer synapses are retained (Zhou and Cline, 1996). In flies that express a CaMKII inhibitory protein, the motor nerve terminals make a greater number of terminal boutons on the muscle cells than in flies lacking the enzyme (Wang et al., 1994). In *C. elegans*, both loss-of-function and gain-of-function unc-43 (CaMKII) mutations exhibit a reduced density of glutamatergic synapses in adults (Rongo and Kaplan, 1999).

The present results show that PKC and PKA activities are also required for inhibitory synaptic depression (Figs. 3 and 4). Previous findings implicate both enzymes in alternate forms of inhibitory synaptic plasticity, although there is no uniform model of action. Our results are most consistent with the "rundown" of $GABA_A$ receptor-mediated chloride currents in cerebellar granule cells, which can be prevented with a PKC inhibitor (Robello et al., 1993). Similarly, the enhancement of GABA receptor-mediated IPSPs in the hippocampus can be induced with PKC antagonists (Xie and Sastry, 1991). In contrast, PKC itself enhances both the amplitude and decay times of mIPSCs in the dentate gyrus, whereas PKA activity is ineffective (Poisbeau et al., 1999). Interestingly, whereas PKC is ineffective in CA1 pyramidal neurons, PKA reduces amplitude and enhances decays of mIPSCs (Poisbeau et al., 1999).

In CA1 hippocampal neurons, both CaMKII and PKA are prominent participants in LTP (Pettit et al., 1994; Blitzer et al., 1998). At mossy fiber synapses, LTP requires elevation of postsynaptic calcium and activation of PKA (Yeckel et al., 1999). The immediate use-dependent history of excitatory synapses determines whether CaMKII or PKA signaling will be favored in the hippocampus. LTD induction of naive synapses dephosphorylates a PKA-sensitive site on AMPA receptors, whereas LTD at potentiated synapses leads to the dephosphorylation of a CaMKII-sensitive site. In contrast, LTP induction at naive or depressed synapses occurs when the CaMKII or PKA sites are phosphorylated, respectively (Lee et al., 2000). Our data supports a role for postsynaptic cAMP and PKA during inhibitory synaptic depression because the antagonists of PKA block depression while enhancement of PKA facilitates depression without the need for afferent activity (Figs. 3 and 4). The latter finding is similar to the depressive effect of cAMP/PKA agonists on GABAergic responses (Xi et al., 1997). There are few manipulations of cAMP signaling during synaptogenesis, but the data suggest that this signaling system promotes synapse formation (Zhong and Wu, 1991).

Although activation of $GABA_B$ and Trk receptors may initiate inhibitory LTD in the LSO (Kotak et al., 2001), the intracellular pathway is not known. Our current findings suggest that CaMKII and PKA are strong candidates for transducing the membrane signal. Our previous work demonstrated a developmental shift in GABAergic and glycinergic transmission (Kotak et al., 1998), and preliminary observations indicate that either $GABA_A$ or glycine receptors can each

contribute to synaptic depression (Chang et al., 2001). Therefore, one scenario that is consistent with the literature on excitatory synaptic plasticity is that CaMKII and/or PKA induce functional alterations at $GABA_A$ and glycine receptors. Such processes may eventually lead to synapse elimination. For example, at the vertebrate neuromuscular junction, prior to withdrawal of some nerve terminals, postsynaptic acetylcholine receptor clusters are lost (Balice-Gordon and Lichtman, 1993).

Intracellular signaling initiated by the activation of $GABA_B$ receptors has been found in other forms of inhibitory plasticity. For example, in the rat visual cortex, $GABA_B$ receptors, calcium, phospholipase C and IP_3 are implicated in inhibitory LTP (Komatsu, 1996; Komatsu and Yoshimura, 2000). A more direct comparison can be drawn between the inhibitory plasticity in cerebellar Purkinje cells and the present results. Bursts of excitatory synaptic activity lead to Ca^{2+}/CaMKII-dependent heterosynaptic potentiation of GABAergic terminals (Kano et al., 1992, 1996), and this potentiation can be blocked by the activity of GABAergic terminals via a postsynaptic $GABA_B$ receptor (Kawaguchi and Hirano, 2000). Therefore, a rise in postsynaptic free calcium produces different effects on inhibition in the two systems: potentiation in the cerebellum and depression in the LSO. However, $GABA_B$ signaling seems to mediate a similar process of synaptic depression in the two systems.

Although we do not have any evidence to demonstrate that $GABA_B$ receptor activation leads to elevation of cAMP or modulation of CaMKII signaling, these are reasonable scenarios. In Figure 5, we propose a model that incorporates the present findings with previous observations on inhibitory plasticity. Our model predicts that GABA and glycine are coreleased by each MNTB terminal and that glycine, $GABA_A$, and $GABA_B$ receptors are each present on the postsynaptic LSO neuron (Kotak et al., 1998). Although $GABA_B$ receptors are thought to be coupled only to G_i (Couve et al., 2000), there is experimental evidence showing that $GABA_B$ receptor activation may activate an adenylate cyclase and PKA, perhaps indirectly (Cunningham and Enna, 1996; Xi et al., 1997). Because inhibitory depression is $GABA_B$ receptor-dependent (Kotak et al., 2001), and our present results indicate that postsynaptic PKA is involved, our preliminary model (Fig. 5) shows $GABA_B$ receptor signaling activating postsynaptic adenylate cyclase and PKA. Alternatively, $GABA_B$ receptor activation could act directly through CREB2 (White et al., 2000). It is possible that excitatory afferents to the LSO increase intracellular calcium and activate CaMKII and PKC. Alternatively, release of neurotrophins from inhibitory terminals and activation of Trks (Kotak et al., 2001) could release calcium from intracellular stores (Kang and Schuman, 2000). In neonatal rat hippocampus, for example, calcium-induced calcium release can lead to LTD of GABAergic transmission (Caillard et al., 2000).

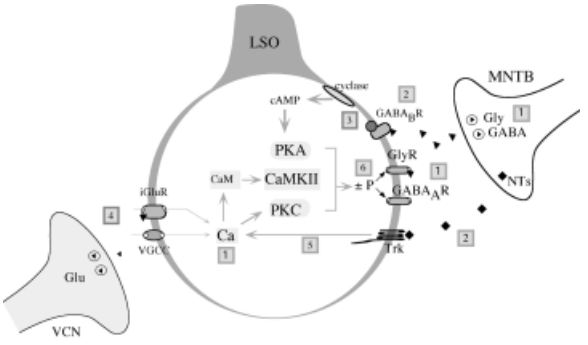

Figure 5 A cellular model of long-term inhibitory synaptic depression at the gerbil MNTB-LSO synaptic contact. See Discussion for details. For reference numbers in the model: (1) Kotak et al., 1998, (2) Kotak and Sanes, 2000; Kotak et al., 2001, (3) Cunningham and Enna, 1996; Xi et al., 1997, (4) Kotak and Sanes, 1995, (5) Kang and Schuman, 2000, (6) Chen et al. 1990; McDonald et al., 1998; Mascia et al., 1998; Poisbeau et al., 1999; Nusser et al., 1999.

Activation of CaMKII and/or PKA/PKC could eventually affect the phosphorylation state of $GABA_A$ or glycine receptors, thereby altering their conductance (Chen et al., 1990; Lin et al., 1996; Mascia et al., 1998; McDonald et al., 1998; Nusser et al., 1999, Poisbeau et al., 1999; Chang et al., 2001). For example, a pioneering study by Chen et al. (1990) demonstrated that phosphorylation (presumably of $GABA_A$ receptors) could prevent the "run-down" of GABA-evoked responses in dissociated hippocampal neurons. These manipulations included exclusion of ATP from the recording solution, inclusion of a hydrolysis-resistant ATP analog in the recording solution, and a decrease of intracellular calcium or calmodulin. Such a "run-down" of IPSCs is analogous to the activity-dependent LTD of IPSCs that we recorded in LSO neurons (Kotak and Sanes, 2000; Fig. 1). It is believed that PKA or PKC can decrease GABA receptor function by phosphorylating the Ser/Thr residues on GABA receptor subunits (Sigel, 1995; Moss and Smart, 1996). In one of the more recent studies, PKC isoforms are shown to bind directly at intracellular domains of $GABA_A$ receptor β subunits to induce phosphorylation and thus modulate synaptic inhibition (Brandon et al., 1999). Similarly, glycine receptors can be modulated by PKC-dependent phosphorylation (Ruiz-Gömez et al., 1991). Overall, in the

developing MNTB-LSO circuit, such cellular processes that weaken inhibitory synaptic strength via participation of several key biochemical pathways at the postsynaptic locus could initiate processes for an eventual loss of inhibitory synaptic terminals, as shown by anatomical observations (Sanes and Siverls, 1991; Sanes and Takács, 1993).

REFERENCES

Balice-Gordon R-J, Lichtman JW. 1993. *In vivo* observations of pre- and postsynaptic changes during the transition from multiple to single innervations at developing neuromuscular junctions. J Neurosci 13:834–855.

Bliss TVP, Lømo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbits following stimulation of the perforant path. J Physiol 232:331–356.

Blitzer RD, Connor JH, Brown GP, Wong T, Shenolikar S, Iyngar R, Landau EM. 1998. Gating of CamKII by cAMP-regulated protein phosphatase activity during LTP. Science 280:1940–1943.

Boulanger L, Poo M-m. 1999. Gating of BDNF-induced synaptic potentiation by cAMP. Science 284:1982–1984.

Brandon NJ, Uren JM, Kittler JT, Wang, H, Olsen R, Parker PJ, Moss SJ. 1999. Subunit-specific association of protein kinase C and the receptor for activated C kinase with GABA type A receptors. J. Neurosci 19:9228–9234.

Caillard O, Ben-Ari Y, Gaiarsa J-L. 2000. Activation of presynaptic and postsynaptic ryanodine-sensitive calcium stores is required for the induction of long-term depression at GABAergic synapses in the neonatal rat hippocampus. J Neurosci 20:RC94(1–6).

Chang E, Kotak VC, Sanes DH. 2001. An evaluation of presynaptic release and GABA signaling in long-term inhibitory synaptic depression in the LSO. Soc Neurosci Abstr 27.

Chen QX, Seltzer A, Kay AR, Wong, RKS. 1990. $GABA_A$ receptor function is regulated by phosphorylation in acutely dissociated guinea-pig hippocampal neurones. J Physiol 420:207–221.

Couve A, Moss SJ, Pangalos MN. 2000. GABAB receptors: a new paradigm in G protein signaling. Mol Cell Neurosci 16:296–312.

Cunningham MD, Enna SJ. 1996. Evidence for pharmacologically distinct $GABA_B$ receptors associated with cAMP production in rat brain. Brain Res 720:220–224.

Davis GW, Schuster CM, Goodman CS. 1996. Genetic dissection of structural and functional components of synaptic plasticity. III. CREB is necessary for presynaptic functional plasticity. Neuron 17:669–676.

Ganguly K, Schinder AF, Wong ST, Poo M-m. 2001. GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell 105:521–532.

Kakazu Y, Akaike N, Komiyama S, Nabekura J. 1999. Regulation of intracellular chloride by cotransporters in developing lateral superior olive neurons. J Neurosci 19:2843–2851.

Kang H, Schuman EM. 2000. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 267:1658–1662.

Kano M, Hashimoto K, Chen C, Abeliovich A, Aiba A, Kurihara H, Tonegawa S. 1995. Impaired synapse elimination during cerebellar development in PKCγ mutant mice. Cell 83:1223–1231.

Kano M, Kano M, Fukunaga K, Konnerth A. 1996. Ca(2+)-induced rebound potentiation of gamma-aminobutyric acid-mediated currents requires activation of Ca2+/calmodulin-dependent kinase II. Proc Natl Acad Sci USA 93:13351–13356.

Kano M, Rexhausen U, Dreessen J, Konnerth A. 1992. Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. Nature 356:601–604.

Kapfer C, Seidl A, Schweizer H, Koch U, Grothe B. 2002. Activity dependent refinement of glycinergic input to medial superior olivary neurons in the gerbil. Nat Neurosci 5:247–253.

Kawaguchi S-y, Hirano T. 2000. Suppression of inhibitory synaptic potentiation by presynaptic activity through postsynaptic GABAB receptors in a Purkinje neuron. Neuron 27:339–347.

Komatsu Y. 1994. Age-dependent long-term potentiation of inhibitory synaptic transmission in rat visual cortex. J Neurosci 14:6488–6499.

Komatsu Y. 1996. $GABA_B$ receptors, monoamine receptors, and postsynaptic inositol triphosphate-induced Ca^{2+} release are involved in the induction of long-term potentiation at visual cortical inhibitory synapses. J Neurosci 16:6342–6352.

Komatsu Y, Yoshimura Y. 2000. Activity-dependent maintenance of long-term potentiation at visual cortical inhibitory synapses. J Neurosci 20:7539–7546.

Kotak VC, Sanes DH. 1995. Synaptically evoked prolonged depolarizations in the developing auditory system. J Neurophysiol 74:1611–1620.

Kotak VC, Sanes DH. 1999. LTD of synaptic inhibition in the superior olive is age and CaMKII-dependent. Soc Neurosci Abstr 25.

Kotak VC, DiMattina C, Sanes DH. 2001. $GABA_B$ and Trk receptor signaling mediates long-lasting inhibitory synaptic depression. J Neurophysiol 86:536–540.

Kotak VC, Korada S, Schwartz IR, Sanes DH. 1998. A developmental shift from GABAergic to glycinergic transmission in the central auditory system. J Neurosci 18:4646–4655.

Lee HK, Barbarosie M, Zkameyama K, Huganir RL, Bear MF. 1998. NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 21:1151–1162.

Lee HK, Barbarosie M, Zkameyama K, Bear MF, Huganir RL. 2000. Regulation of distinct AMPA receptor phos-

phorylation sites during bidirectional synaptic plasticity. Nature 405:955–959.

Lin Q, Peng, YB, Willis WD. 1996. Inhibition of primate spinothalamic tract neurons by spinal glycine and GABA is reduced during central sensitization. J Neurophysiol 76:1005–1014.

Linden DJ, Connor JA. 1995. Long-term synaptic depression. Annu Rev Neurosci 18:319–357.

Mascia MP, Wick MJ, Martinez LD, Harris RA. 1998. Enhancement of glycine receptor function by ethanol: role of phosphorylation. Br J Pharmacol 125:263–270.

Madison DV, Malenka RA, Nicoll RA. 1991. Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 14:379–397.

McDonald BJ, Amato A, Connolly CN, Benke D, Moss SJ, Smart TG. 1998. Adjacent phosphorylation sites on GABAA receptor beta subunits determine regulation by cAMP-dependent protein kinase. Nat Neurosci 1:123–128.

Morishita W, Sastry BR. 1991. Chelation of postsynaptic Ca^{2+} facilitates long-term potentiation of hippocampal IPSPs. Neuroreport 2:533–536.

Moss SJ, Smart TG. 1996. Modulation of amino acid-gated ion channels by protein phosphorylation. Int Rev Neurobiol 39:1–52.

Mulkey RM, Malenka RC. 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Nusser Z, Sieghart W, Mody I. 1999. Differential regulation of synaptic $GABA_A$ receptors by cAMP-dependent protein kinase in mouse cerebellar and olfactory bulb neurones. J Physiol 2:421–435.

Oda Y, Charpier S, Murayama Y, Suma C, Korn H. 1995. Long-term potentiation of glycinergic inhibitory synaptic transmission. J Neurophysiol 74:1056–1074.

Oda Y, Kawasaki K, Morita M, Korn H, Matsui H. 1998. Inhibitory long-term potentiation underlies auditory conditioning of goldfish escape behavior. Nature 394:182–185.

Pettit DL, Perlman S, Malinow R. 1994. Potentiated transmission and prevention of further LTP by increase CaMKII activity in postsynaptic hippocampal slice neurons. Science 266:1881–1884.

Poisbeau P, Cheney MC, Browning MD, Mody I. 1999. Modulation of synaptic $GABA_A$ receptor function by PKA and PKC in adult hippocampal neurons. J Neurosci 19:674–683.

Robello M, Amico C, Cupello A. 1993. Regulation of $GABA_A$ receptor in cerebellar granule cells in culture: differential involvement of kinase activities. Neuroscience 53:131–138.

Rongo C, Kaplan JM. 1999. CaMKII regulates the density of central glutamatergic synapses in vivo. Nature 402: 195–199.

Ruiz-Gomez A, Vaello ML, Valdivieso F, Mayor F Jr. 1991. Phosphorylation of the 48-KD2 subunit of the glycine receptor by protein kinase C. J Biol Chem 266: 559–566.

Sanes DH, Friauf E. 2000. Review: development and influence of inhibition in the lateral superior olivary nucleus. Hear Res 147:46–58.

Sanes DH, Siverls V. 1991. The development and specificity of inhibitory axonal arborizations in the lateral superior olive. J Neurobiol 22:837–854.

Sanes DH, Takács C. 1993. Activity-dependent refinement of inhibitory connections. Eur J Neurosci 5:570–574.

Sanes DH, Reh TA, Harris WA. 2000. Development of the nervous system. New York: Academic Press.

Shira TS, Pearson HA. 1995. Ca/Calmodulin-dependent kinase II inhibitor KN62 attenuates glutamate release by inhibiting voltage-dependent ca(2+)-channels. Neuropharmacology 34:731–741.

Sigel E. 1995. Functional modulation of ligand-gated $GABA_A$ and NMDA receptor channles by phosphorylation. J Recept Signal Trasduc Res 15:325–333.

Sun ZY, Schacher S. 1996. Tetanic stimulation and cyclic adenosine monophosphate regulate segregation of presynaptic inputs on a common postsynaptic target neuron in vitro. J Neurobiol 29:183–201.

Vale C, Sanes DH. 2000. Afferent regulation of inhibitory synaptic transmission in the developing auditory midbrain. J Neurosci 20:1912–1921.

Wang F, Renger JJ, Griffith LC, Greenspan RJ, Wu CF. 1994. Concomitant alterations of physiological and developmental plasticity in Drosophila CaM kinase II-inhibited synapses. Neuron 13:1373–1384.

White JH, McIllhinney RA, Wise A, Ciruela F, Chan WY, Emson PC, Billinton A, Marshall FH. 2000. The GABAB receptor interacts directly with the related transcription factors CREB2 and ATFx. Proc Natl Acad Sci USA 97:13967–13972.

Xi ZX, Yamada K, Tsurusaki M, Akasu T. 1997. Baclofen reduces GABAA receptor responses in acutely dissociated neurons of bullfrog dorsal root ganglia. Synapse 26:165η174.

Xie Z, Sastry BR. 1991. Inhibition of protein kinase activity enhances long-term potentiation of hippocampal IPSPs. Neuroreport 2:389–392.

Yeckel MF, Kapur A, Johnston D. 1999. Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. Nat Neurosci 2:625–633.

Zhong Y, Wu CF. 1991. Altered synaptic plasticity in *Drosophila* memory mutants with a defective cyclic AMP cascade. Science 251:198–201.

Zou D-J, Cline HT. 1996. Expression of constitutively active CaMKII in target tissue modifies presynaptic axon arbor growth. Neuron 16:529–539.