$GABA_B$ and Trk Receptor Signaling Mediates Long-Lasting Inhibitory Synaptic Depression

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Kotak, Vibhakar C., Christopher DiMattina, and Dan H. Sanes. $GABA_B$ and Trk receptor signaling mediates long-lasting inhibitory synaptic depression. *J Neurophysiol* 86: 536–540, 2001. In many areas of the nervous system, excitatory and inhibitory synapses are reconfigured during early development. We have previously described the anatomical refinement of an inhibitory projection from the medial nucleus of the trapezoid body to the lateral superior olive in the developing gerbil auditory brain stem. Furthermore, these inhibitory synapses display an age-dependent form of long-lasting depression when activated at a low rate, suggesting that this process could support inhibitory synaptic refinement. Since the inhibitory synapses release both glycine and GABA during maturation, we tested whether $GABA_B$ receptor signaling could initiate the decrease in synaptic strength. When whole cell recordings were made from lateral superior olive neurons in a brain slice preparation, the long-lasting depression of medial nucleus of the trapezoid body–evoked inhibitory potentials was eliminated by the $GABA_B$ receptor antagonist, SCH-50911. In addition, inhibitory potentials could be depressed by repeated exposure to the $GABA_B$ receptor agonist, baclofen. Since $GABA_B$ receptor signaling may not account entirely for inhibitory synaptic depression, we examined the influence of neurotrophin signaling pathways located in the developing superior olive. Bath application of brain-derived neurotrophic factor or neurotrophin-3 depressed evoked inhibitory potentials, and use-dependent depression was blocked by the tyrosine kinase antagonist, K-252a. We suggest that early expression of GABAergic and neurotrophin signaling mediates inhibitory synaptic plasticity, and this mechanism may support the anatomical refinement of inhibitory connections.

INTRODUCTION

Although neuronal discharge can be quite low during early development, spontaneous and evoked activity has a profound impact on the selective loss or survival of synaptic contacts (Sanes et al. 2000a). Manipulations of excitatory transmission can disrupt the normal elimination of motor axons onto muscle fibers, and prevent the refinement of excitatory connections in the CNS (Cline et al. 1987; Ichise et al. 2000; Kleinschmidt et al. 1987; O'Brien et al. 1978; Scherer and Udin 1989; Simon et al. 1992; Thompson et al. 1979). There is now evidence that inhibitory terminals also become refined during development. In the gerbil lateral superior olive (LSO), the inhibitory afferent fibers from the medial nucleus of the trapezoid body (MNTB) become restricted anatomically during postnatal development (Sanes and Siverls 1991).

Stimulation of MNTB afferents at a low rate leads to a long-lasting depression of synaptic inhibition in LSO neurons (Kotak and Sanes 2000). This form of inhibitory synaptic plasticity declines with age, and we have hypothesized that it contributes to the activity-dependent reorganization of MNTB arbors within the LSO (Sanes and Takács 1993). Although long-term inhibitory synaptic depression has been reported in this and other systems (Komatsu 1994; Morishita and Sastry 1991; Oda et al. 1998), the signaling pathway that initiates this form of plasticity has not been examined. In contrast, co-activation of glutamatergic and GABAergic afferents can produce inhibitory depression through an *N*-methyl-D-aspartate (NMDA) receptor mechanism (Caillard et al. 1999).

This present study focuses on two candidate signaling systems. First, the MNTB-evoked inhibitory response recorded in the gerbil LSO is predominantly GABAergic before hearing onset and switches to a predominantly glycinergic input postnatally (Kotak et al. 1998). This finding suggested that GABAergic transmission could play a significant role during inhibitory synaptogenesis. Second, MNTB neurons express neurotrophins, and LSO neurons express their cognate receptors during development (Hafidi 1999; Hafidi et al. 1996). Since neurotrophin/Trk signaling pathways have been shown to modulate synaptic transmission (Kang and Schuman 1995; Kim et al. 1994; Levine et al. 1998), they may be relevant to the plasticity displayed by MNTB synapses. Therefore we have tested whether signals mediated by $GABA_B$ and neurotrophin receptors are involved in the long-lasting depression of inhibitory synapses in the LSO.

METHODS

Gerbils (*Meriones unguiculatus*) aged *postnatal days 8–12* (*P8–12*) were used to make 300-μM coronal brain slices through the LSO and MNTB. The artificial cerebrospinal fluid (ACSF) contained (in mM) 125 NaCl, 4 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 26 NaHCO3, 15 glucose, 2.4 $CaCl_2$, and 0.4 L-ascorbic acid (pH 7.3 when bubbled with 95% O_2-5% CO_2). The ACSF was continuously superfused in the recording chamber at 4–5 ml per min at room temperature (22–24°C). Whole cell current-clamp recordings were obtained from LSO neurons (Warner PC-501A), and 200-μs electrical pulses were delivered directly to the MNTB, as described previously (Kotak and Sanes 2000). The internal patch solution contained (in mM) 127.5 potassium gluconate, 0.6 EGTA, 10 HEPES, 2 $MgCl_2$, 5 KCl, 2 ATP, and 0.3 GTP (pH 7.2). To block tyrosine kinase in the postsynaptic LSO

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neuron, K-252a (200 nM) was added to the internal pipette solution. To examine inhibitory synaptic depression, MNTB-evoked maximum amplitude inhibitory postsynaptic potentials (IPSPs) were first acquired during a 15-min baseline period initially every minute for the first 5 min and then at the 10th and 15th min (Kotak and Sanes 2000). The MNTB was then activated with low-frequency stimulation (LFS: 1 Hz for 15 min). Immediately following LFS, MNTB-evoked IPSPs were recorded every min for the first 5 min and every 5 min thereafter. To block $GABA_B$ receptors, SCH-50911 (5–10 μM, Tocris) was bath-applied throughout the experiment beginning 5 min before recording the first IPSP.

In a separate set of experiments, IPSPs were recorded for about 1 h at a very low rate of acquisition that does not produce synaptic depression (0.03 Hz), and the slices were exposed to either a $GABA_B$ receptor agonist (baclofen, 100 μM, Sigma Chemicals), or a neurotrophin [brain derived neurotrophic factor (BDNF), 50–100 ng/ml, Sigma Chemicals or Alamone Laboratories; NT-3, 25–50 ng/ml, Sigma Chemicals]. In many of these experiments, contaminating glutamatergic activity was blocked with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) or kynurenic acid (4 mM). This was done for control LFS experiments ($n = 3$), baclofen exposure ($n = 2$), BDNF exposure ($n = 7$), NT-3 exposure ($n = 6$), and SCH-50911 treatment ($n = 3$).

Data were collected using a Macintosh PPC running a custom-designed IGOR (WaveMetrics, v3.14) macro called SLICE. The data were analyzed off-line using a second IGOR macro called SLICE ANALYSIS. Each macro is available with complete documentation on-line at http://www.cns.nyu.edu/~sanes/slice_software. The SLICE macro controls the stimulus isolation units and patch-clamp amplifier via an ITC-18 Computer Interface (Instrutech Corporation) using an IGOR external operation commands (XOP version 2.6, Instrutech). Data were sampled and stored at 10 kHz. Analyses of peak IPSP amplitude, rising slope, and duration were performed off-line. Data are presented as means ± SE or as a percent of the normalized IPSP amplitudes as indicated in RESULTS and the figure legends. All analyses were performed with the Student's *t*-test.

RESULTS

The data reported here are drawn from whole cell current-clamp recordings from 74 LSO neurons. Each recording was obtained from a separate brain slice. In the initial experiments, MNTB-evoked maximum amplitude IPSPs were recorded without any pharmacological agents in the ACSF. As shown for a control *P9* neuron in Fig. 1, the MNTB-evoked IPSP was about 11 mV during the pre-LFS period, but decreased to about 6.5 mV following LFS treatment (*top*). The average IPSP amplitude reduction was 43% at 1 h following LFS, as compared with the baseline IPSP amplitude prior to LFS ($n = 10$). In three recordings, ionotropic glutamate receptors were blocked with kynurenic acid (4 mM), and this did not alter the magnitude of depression (a 45% reduction in IPSP amplitude was observed). To assess the role of $GABA_B$ receptors during the initiation of inhibitory synaptic depression, we applied the $GABA_B$ receptor antagonist SCH-50911 (5–10 μM) throughout the experiment, beginning 5 min before the first IPSP was recorded. As shown in Fig. 1, when LFS was delivered in the presence of SCH-50911, the magnitude of long-lasting depression was blocked as compared with the untreated controls.

The second experimental strategy to assess $GABA_B$ receptor involvement in inhibitory depression was an extension of our previous finding that baclofen reversibly depressed IPSPs following a single exposure (Kotak et al. 1998). As shown in Fig. 2, repeated perfusion (100 μM baclofen; 5 × 10 s exposures at

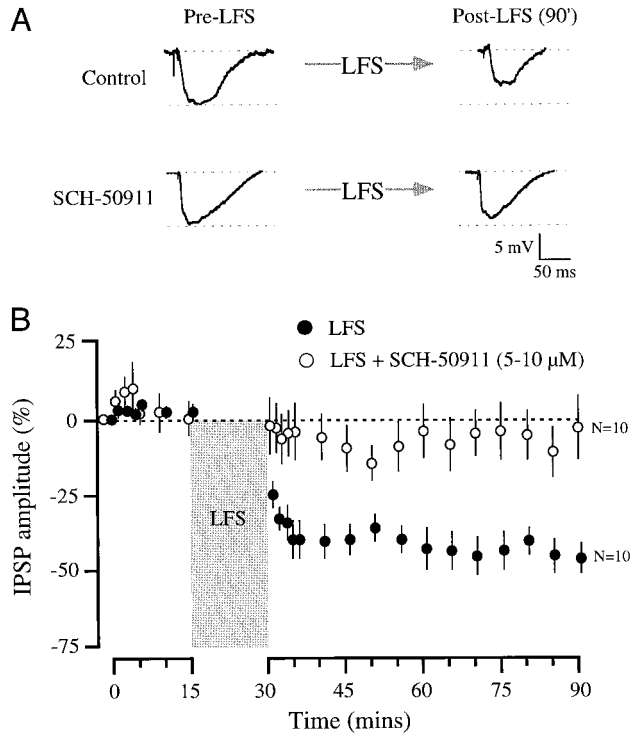

FIG. 1. Long-lasting depression of inhibitory transmission was mediated by $GABA_B$ receptors. *A*: medial nucleus of the trapezoid body (MNTB)–evoked maximum inhibitory postsynaptic potentials (IPSPs) were recorded from the lateral superior olive (LSO) before and after low-frequency stimulation (LFS) of the MNTB. Example IPSPs are shown for a *postnatal day 9* (*P9*) neuron recorded in control (*top*) or SCH-5091–containing ACSF (*bottom*). E_{rest} was −53 and −52 mV, respectively. *B*: summary for all recorded LSO neurons at *P8–12* in the absence and presence of SCH-50911 (mean ± SE). Synaptic depression was robust (43%) at 50–60 min following LFS when compared with pre-LFS IPSPs (●). Age-matched neurons treated with SCH-50911 (○) displayed a marginal change in IPSP amplitude following LFS. The mean percent change was calculated by comparing the average normalized IPSP amplitude recorded at 50–60 min post LFS with the normalized mean initial IPSP amplitude (0%) during 1st 5 min of the recording session (for control neurons: $t = 5.1$, df = 18, $P < 0.0001$; for SCH-50911–treated neurons: $t = -0.56$, df = 18, $P = 0.57$).

3-min intervals) induced a long-lasting depression. There was also a significant decrease in the IPSP rising slope (50% decline, $P < 0.01$). In three of four neurons tested, the LSO input resistance decreased by approximately 30% during baclofen exposure. In two additional experiments, a single dose exposure of baclofen (100 μM) caused the MNTB-evoked IPSPs to decrease by about 50% for approximately 10 min. This baclofen-elicited depression was eliminated when the slice was pretreated for 6 min with 10 μM SCH-50911 (data not shown). This indicated that the synaptic- and agonist-mediated depression involved the same receptor.

While the $GABA_B$ receptor antagonist results suggest that this receptor is necessary for induction of inhibitory depression, additional mechanisms have not been ruled out. Therefore two neurotrophin signaling systems (BDNF and NT-3) known to be localized to the MNTB-LSO pathway were tested as candidates for a depression mechanism. For these experiments, IPSPs were recorded every 30 s for approximately 1 h. In control recordings, this stimulus rate did not alter IPSP amplitude significantly. The change in IPSPs was calculated by comparing the mean IPSP amplitude (±SE) recorded at 50–60 min with the mean initial IPSP amplitude (±SE) during first 10

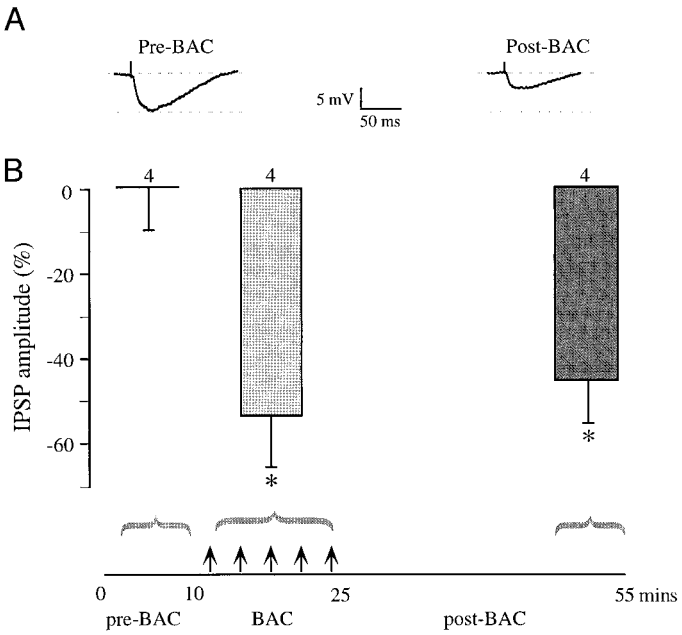

FIG. 2. Repeated activation of $GABA_B$ receptors elicited long-lasting synaptic depression. *A*: a maximum amplitude IPSP is shown for a *P10* neuron before and after repeated baclofen exposure. *B*: the bar graph compares the percent change in the normalized IPSP amplitude during a control period, a 15-min drug exposure period, and after a recovery period. There was a significant decline (asterisk) in IPSP amplitudes during baclofen treatment, and this depression persisted at 30 min after the last baclofen exposure. The change in IPSPs was calculated by comparing the average normalized IPSP amplitude recorded during baclofen exposure and at 50–60 min of the experiments with the initial IPSP amplitude (0%) during 1st 10 min of the recording session (comparison between initial IPSPs and IPSPs during baclofen exposure: $t = 4.01$, df = 6, $P < 0.007$; comparison between initial IPSPs and IPSPs at 50–60 min: $t = 4.08$, df = 6, $P < 0.006$).

min of the recording session (initial IPSP amplitude = 8.7 ± 0.7 mV, mean ± SE; IPSP amplitude at 50–60 min following LFS = 8.9 ± 0.8 mV; $t = -0.51$, df = 10, $P = 0.620$). In separate recordings, bath application of BDNF (50–100 ng/ml) for 5–8 min resulted in a small decrease in IPSP amplitude. Approximately 10 min after BDNF application, IPSP amplitudes declined, and this attenuation reached its maximum by about 20–30 min following drug exposure, but the change did not reach significance (comparison between initial IPSPs and IPSPs during BDNF exposure: $t = 0.36$, df = 18, $P = 0.72$; comparison between initial IPSPs and IPSPs at 50–60 min: $t = 0.17$, df = 14, $P = 0.07$). Exposure to NT-3 (25–50 ng/ml) produced a larger and more rapid decline in IPSP amplitude, and this decline was highly significant (Fig. 3). Finally, to assess whether neurotrophin receptors could influence synaptically evoked depression, a tyrosine kinase antagonist (200 nM K-252a) was added to the internal patch solution. As shown in Fig. 3*B*, K-252a prevented LFS from inducing a significant change in IPSP amplitude (mean initial IPSP amplitude = 9 ± 1 mV; mean IPSP amplitude at 50–60 min following LFS = 9.7 ± 0.2 mV).

DISCUSSION

A number of studies suggest that auditory coding properties mature postnatally, and that this improvement is due, in part, to experience-dependent mechanisms (Sanes and Walsh 1997). For example, sound localization in the barn owl is influenced by both auditory and visual experience (Knudsen and Brainard 1991; Mogdans and Knudsen 1993). In the gerbil LSO, interaural level difference coding improves with age, and several anatomical and physiological properties are disrupted by deafferentation during development (Sanes et al. 2000b). We have previously shown that inhibitory projections from MNTB to LSO become refined during development, and this process is disrupted by deafferentation (Sanes and Siverls 1991; Sanes and Takács 1993). More recently, we have found that the strength of these inhibitory synapses depends on activity, and this phenomenon wanes with age (Kotak and Sanes 2000). The present results suggest that use-dependent depression of inhibitory synapses requires $GABA_B$ receptors, and may also employ neurotrophin signaling.

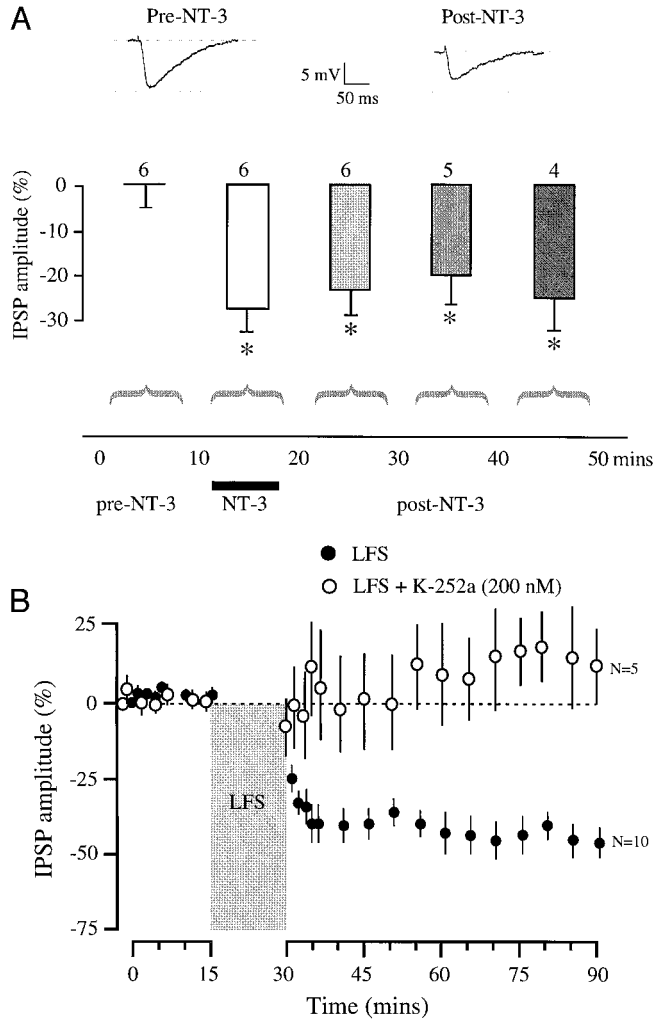

FIG. 3. Neurotrophin signaling depresses inhibitory transmission. *A*: a maximum amplitude IPSP is shown for a *P11* neuron before and after NT-3 (25 ng/ml) exposure for 8 min. The IPSP depressed by about 30%. The bar graph compares percent change in IPSP amplitude before, during, and after NT-3 exposure. The IPSPs decreased significantly during NT-3 application when compared with pre-NT-3 treatment IPSPs (asterisk), and remained depressed at 40–50 min (comparison between initial IPSPs and IPSPs during NT-3 exposure: $t = 2.58$, df = 10, $P = 0.02$; comparison between initial IPSPs and IPSPs at 50–60 min: $t = 4.65$, df = 8, $P = 0.001$). *B*: summary for neurons in the absence and presence of K-252a (control data from Fig. 1). Neurons recorded with K-252a in the pipette solution (○) displayed no change in IPSP amplitude following LFS (comparison between initial IPSPs and IPSPs at 50–60 min: *t*-test; $t = -0.73$, df = 8, $P = 0.48$).

Inhibitory synapses in LSO are predominantly GABAergic during the first two postnatal weeks, and gradually adopt a glycinergic phenotype (Kotak et al. 1998). This led us to hypothesize that GABA may provide a metabotropic signal that is important for synapse maturation. In the present study, we found that blockade of $GABA_B$ receptor transduction could eliminate long-lasting synaptic depression (Fig. 1). This result is consistent with the ability of a $GABA_B$ agonist to initiate long-lasting depression (Fig. 2). While it is not yet clear how $GABA_B$ receptor activation initiates inhibitory depression, a G protein–linked mechanism has recently been shown to depress $GABA_A$ receptor–gated responses through alteration of cytoskeletal anchoring proteins (Meyer et al. 2000). Postsynaptic $GABA_B$ receptors apparently exist in LSO since these neurons exhibited an increased conductance following baclofen exposure. However, a presynaptic contribution to inhibitory depression cannot be ruled out. For example, presynaptic $GABA_B$ receptor–coupled mechanisms are known to decrease transmission at both excitatory and inhibitory synapses (Brenowitz et al. 1998; Lim et al. 2000; Takahashi et al. 1998). However, these effects commonly last for seconds to minutes and are not as likely to underlie the long-lasting change we observe in the LSO.

Neurotrophins and their receptors have also been implicated in synapse development and plasticity. In cerebellar cultures, activity blockade reduces the number of inhibitory synapses, but inhibitory synaptogenesis is restored by BDNF or neurotrophin-4 (NT-4), while antibodies to BDNF and NT-4 reduce inhibitory synapse formation (Seil and Drake-Baumann 2000). In addition, NT-3 depresses $GABA_A$ receptor–mediated transmission in developing cortical neurons (Kim et al. 1994). In the MNTB-LSO pathway, immunoreactivity for BDNF, NT-3, and their receptors is quite prominent during the first two postnatal weeks (Hafidi 1999; Hafidi et al. 1996). In the present study, neurotrophin-3 exposure depressed inhibitory synaptic gain (Fig. 3*A*). IPSP amplitude declined within 10 min of exposure, but this slow time course may have been due to access to the recording site within the brain slice. The blockade of use-dependent depression by K-252a implies that neurotrophin receptors may participate along with $GABA_B$ receptors to induce inhibitory depression. One possibility is that the neurotrophin signal acts to raise intracellular free calcium (Kang and Schuman 2000), which is required for inhibitory depression to occur in LSO neurons (Kotak and Sanes 2000). As in excitatory synaptogenesis, adjustments of inhibitory synaptic strength may thus be regulated by several receptors and intracellular signaling pathways. Dissection of those mechanisms will be critical to appreciate the functionality of inhibitory synapses before and after sound-evoked activity (Kotak and Sanes 2000).

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