Brain-Derived Neurotrophic Factor (BDNF) Modulates Inhibitory, But Not Excitatory, Transmission in the CA1 Region of the Hippocampus

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Frerking, M., R. C. Malenka, and R. A. Nicoll. Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. J. Neurophysiol. 80: 3383 ± 3386, 1998. Brain-derived neurotrophic factor (BDNF) has been reported to have rapid effects on synaptic transmission in the hippocampus. We report here that bath application of BDNF causes a small but significant decrease in stimulus-evoked inhibitory postsynaptic currents (IPSCs) on CA1 pyramidal cells, which is prevented by the tyrosine kinase inhibitor lavendustin A. BDNF decreases in the 1/CV^2 of the IPSC, and also reduces paired-pulse depression of the IPSC, suggesting a presynaptic site of action. In contrast, BDNF did not have a detectable effect on field excitatory postsynaptic potentials measured in stratum radiatum. We conclude that BDNF has a selective depressant action on inhibitory transmission in the hippocampus, due at least in part to a presynaptic mechanism.

METHODS

Slice preparation and recording techniques

Hippocampal slices 500 μm thick were prepared from 2- to 8-wk-old Sprague-Dawley rats as described (Wyllie et al. 1994). After >1 h recovery time, slices were transferred to a submersion-of BDNF causes a small but significant decrease in the 1/CV^2 of the IPSC, and also reduces paired-pulse depression of the IPSC, suggesting a presynaptic site of action. In contrast, BDNF did not have a detectable effect on field excitatory postsynaptic potentials measured in stratum radiatum. We conclude that BDNF has a selective depressant action on inhibitory transmission in the hippocampus, due at least in part to a presynaptic mechanism.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in numerous aspects of development in the CNS. In addition to these presumably chronic actions of BDNF, several recent reports have suggested that BDNF can have rapidly relative effects on synaptic transmission in the CA1 region of the hippocampus, causing an increase in excitatory synaptic transmission (Kang and Schuman 1995, 1996; Kang et al. 1996, 1997) and a decrease in inhibitory synaptic transmission (Tanaka et al. 1997). However, the effects of BDNF on excitatory synaptic transmission have been controversial with several groups reporting minimal or no effects (Figurov et al. 1996; Gottschalk et al. 1998; Patterson et al. 1996; Tanaka et al. 1997). This controversy may be related at least in part to the fact that BDNF penetration into the hippocampal slice is limited (Kang et al. 1996; Patterson et al. 1996).

We examine here the effects of BDNF on synaptic transmission in the CA1 region of the hippocampus. We find that BDNF causes a rapid, tyrosine kinase-dependent decrease in the amplitude of stimulus-evoked inhibitory postsynaptic currents (IPSCs). This action is accompanied by a decrease in the 1/CV^2 of IPSCs, and alters short-term plasticity. However, BDNF had no effect on excitatory synaptic transmission. We conclude that BDNF selectively decreases inhibitory transmission in the hippocampus, at least in part through a presynaptic mechanism.

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METHODS

Slice preparation and recording techniques

Hippocampal slices 500 μm thick were prepared from 2- to 8-wk-old Sprague-Dawley rats as described (Wyllie et al. 1994). After >1 h recovery time, slices were transferred to a submersion-type recording chamber perfused at room temperature with a solution consisting of (in mM) 119 NaCl, 2.62 NaHCO3, 11 glucose, 2.5 KCl, 2.5 CaCl2, 1.3 MgSO4, and 1.0 NaH2PO4, bubbled with 95% O2-5% CO2. Patch electrodes (2–5 MΩ) were filled with a solution adjusted to pH 7.2, 270–290 mosm containing (in mM) 117.5 Cs gluconate, 10 tetrathylammonium chloride (TEACl), 10 N-2-hydroxyethylpiperazine-N,N,N′,N′-tetraacetic acid (EGTA), in all experiments on inhibitory synaptic currents, ionotropic glutamate receptors were blocked with 1 μM 3-(CR)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and 5 μM 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX). Stimulation and whole cell recording techniques were similar to those described in Wyllie et al. (1994). Whole cell patch-clamp recordings from pyramidal cells were made by placing the electrode in stratum pyramidale and blindly advancing the pipette until the patch pipette resistance increased. Stimulus-evoked whole cell currents and voltages were evoked every 20 s and were filtered at 2 kHz and digitized at 5 kHz. Data were analyzed on-line using the Student’s t-test, and correlations were assessed using the Pearson Product Moment Correlation. Significance was assessed at P < 0.05. IPSC amplitudes were calculated by subtracting a baseline period preceding stimulation from a region of 5–10 ms during the peak of the IPSC. Paired-pulse amplitudes were obtained by subtracting the amplitude immediately before the second stimulus from the peak amplitude of the second stimulus. 1/CV^2 was calculated without noise subtraction, because noise under these conditions contributes <5% of the total variance.

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BDNF preparation and handling

BDNF was prepared in phosphate buffer stock solutions and kept at 4°C. The perfusion system was made from chemically inert materials (silicon tubing and a Teflon beaker). BDNF was applied at a flow rate of 200–280 ml/h. BDNF was obtained from several
IPSC (Fig. 1C1), although this effect was highly variable and in some cases could be as large as 50–60% (Fig. 1C2). This effect of BDNF was independent of the holding potential of the postsynaptic cell (data not shown).

To ensure that this small decrease in the IPSC was dependent on BDNF function, which is known to be mediated by tyrosine kinase activation, we compared the effects of BDNF application in the presence and absence of the broad-spectrum tyrosine kinase inhibitor lavendustin A (5 μM). As shown in Fig. 1C, preincubation with lavendustin A completely prevented the effect of BDNF application ($P = 0.002$), confirming a requirement for tyrosine kinase activity.

The small size of the BDNF-induced reduction in IPSC size precludes a detailed analysis of its mechanism, but we briefly explored the locus of action of BDNF on inhibitory transmission in two ways. First, we examined the $1/CV^2$ (mean$^2$/variance) of the IPSC, which is correlated with the number of quanta per stimulus that contribute to the IPSC. We found that the $1/CV^2$ significantly decreased upon BDNF application, and that moreover this decrease was sig-

**RESULTS**

Stimulus-evoked IPSCs in CA1 pyramidal cells were recorded in the presence of the non-N-methyl-D-aspartate (NMDA) receptor antagonist NBQX and the NMDA receptor antagonist CPP. In agreement with the results of Tanaka et al. (1997), bath application of 4–10 nM BDNF caused a small but significant decrease in the size of the stimulus-evoked IPSC ($n = 13$). A typical example is shown in Fig. 1A for averaged IPSCs. The kinetics of the IPSC were not affected by BDNF, and BDNF application was not accompanied by changes in input resistance or holding current (not shown). This effect had a rapid onset and did not show an obvious recovery, as shown in a representative experiment (Fig. 1B) and in an average of all 13 experiments (Fig. 1C). On average, BDNF caused an ~20% decrease in the IPSC (Fig. 1C1), which was not observed in the presence of lavendustin A (5 μM) (Fig. 1C2). This effect of BDNF was independent of the holding potential of the postsynaptic cell (data not shown).

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**FIG. 1.** Brain-derived neurotrophic factor (BDNF) causes a tyrosine kinase–dependent decrease in inhibitory postsynaptic current (IPSC) amplitude. A: averaged, stimulus-evoked IPSCs are compared before and after application of 4 nM BDNF. Holding potential for this cell was 0 mV. B: IPSC amplitude decreases rapidly after bath application of BDNF in a single experiment. C: effects of BDNF are blocked by the tyrosine kinase inhibitor lavendustin A. C1: on average, BDNF causes a significant decrease in IPSC amplitude in the absence ($●$, $n = 13$) but not the presence ($○$, $n = 8$) of lavendustin A. C2: results from individual experiments are shown for both groups in C1, measured 10 min after BDNF application.

**FIG. 2.** BDNF causes a reduction in the probability of release. A: BDNF causes a decrease in the $1/CV^2$ which is correlated with the decrease in mean amplitude. Average ($●$) and individual ($○$) experiments are shown. B: short-term plasticity is altered by BDNF. Paired stimuli 50 ms apart cause a depression of the 2nd response relative to the 1st. This depression is reduced by BDNF, as shown by scaling the 1st response in BDNF to that in control conditions. C: an average of BDNF-induced changes in paired-pulse modulation from 4 cells. All cells studied showed a larger normalized 2nd pulse after BDNF application.
Field excitatory postsynaptic potentials (EPSPs) are not affected by BDNF application. A1: average field EPSPs are shown before (a), during (b), and after bath application of 4 nM BDNF (c). A2: traces from A1 are superimposed for comparison. B: an average of field EPSP slope measurements from 11 slice experiments is shown. Only 1 of these experiments showed a >20% increase in field EPSP slope.

The observation that BDNF has a significant effect on IPSCs provides us with evidence that under our experimental conditions BDNF penetrates the hippocampal slice sufficiently to affect synaptic transmission. We therefore next examined the effects of BDNF on excitatory synaptic transmission in stratum radiatum using field potential recordings, because results in this area have been controversial (see INTRODUCTION). We found no effect of BDNF on field EPSPs in slices from young animals (n = 7; not shown). To replicate more closely the conditions used in those studies where BDNF has been reported to have an effect on excitatory transmission, we repeated these experiments in slices from young adult rats (6–8 wk). We found an increase in field EPSP slope of >20% on BDNF application in only 1 of 11 experiments under these conditions; on average, BDNF had no significant effect on excitatory transmission in our hands (Fig. 3).

**DISCUSSION**

We have presented evidence for a small but rapid depressant effect of the neurotrophin BDNF on inhibitory transmission in the hippocampus, in general agreement with the results of Tanaka et al. (1997). We further contribute the new findings that this effect of BDNF is blocked by the tyrosine kinase inhibitor lavendustin A and is associated with a decrease in the number of quanta contributing to the IPSC and changes in short-term plasticity of the synapse. These two findings are most readily compatible with a presynaptic locus for the effects of BDNF that we observe.

In our hands, the effects of BDNF on synaptic transmission in the hippocampus are modest. The effect that we observe on inhibitory transmission is considerably smaller than that seen by Tanaka et al. (1997), and we observe no consistent effect on excitatory transmission, contrary to several published reports (Kang and Schuman 1995, 1996; Kang et al. 1996, 1997) but in agreement with results from other labs (Figuero et al. 1996; Gottschalk et al. 1998; Patterson et al. 1996; Tanaka et al. 1997). The reason for this discrepancy is not clear, but the limited penetration of BDNF into the slice has been raised as a possible explanation (Kang et al. 1996). Differences in a number of subtle experimental variables might therefore conceivably contribute to the different results, although we have tried to replicate the recording conditions of Kang and Schuman (1995) as exactly as possible, using rapid (>200 ml/h) perfusion, chemically inert perfusion equipment, similarly aged rats, and similar concentrations of BDNF and durations of application. Moreover, we have varied recording depth in an attempt to record from synapses closer to the surface of the slice, without obvious effects on our results (not shown). Finally, the observation that BDNF causes a tyrosine kinase–dependent depression of IPSCs indicates that BDNF in our hands is active and does penetrate the slice at least to some extent. We are therefore left without an obvious explanation for the different results between labs.
Neurotrophins bind to tyrosine kinase–coupled neurotrophin receptors of the Trk class. We have used the tyrosine kinase inhibitor lavendustin A to confirm the involvement of tyrosine kinases in the disinhibitory effect of BDNF. Lavendustin A is known to block the downstream effects of Trk receptor activation (Berg et al. 1995; Contreras 1993); however, to our knowledge a direct effect of lavendustin A on the tyrosine kinase activity intrinsic to Trk receptors has not been studied. We can therefore conclude that tyrosine kinase activity is required for the effects of BDNF shown here, but it is not clear whether lavendustin A inhibits the TrkB receptor directly or some other tyrosine kinase downstream of Trk activation.

Our results suggest that BDNF has a presynaptic locus of action at the interneuron-CA1 synapse. This is somewhat surprising, given the observations that postsynaptic bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA) has been reported to block the effects of BDNF, and that BDNF decreases the responses of CA1 cells to exogenously applied γ-aminobutyric acid (GABA) (Tanaka et al. 1997). The first of these observations is compatible with presynaptic modulation if BDNF generates a retrograde messenger; however, we cannot explain a decrease in GABA sensitivity in presynaptic terms. We suggest that both sets of results could be explained if BDNF has both pre- and postsynaptic effects. Alternatively, the basic observations seen here and in Tanaka et al. (1997) may have different mechanisms, because the TrkB receptors thought to mediate BDNF’s effects are found in both pyramidal and nonpyramidal neurons in the hippocampus (Altar et al. 1994; Marty et al. 1996b). Further elucidation will be of interest.

We have found that BDNF causes a specific reduction of inhibitory transmission in area CA1 of the hippocampus. This modulatory role of BDNF on inhibitory transmission is of particular interest, because BDNF is known to alter interneuron phenotype (Jones et al. 1994; Marty et al. 1996b; Nawa et al. 1994). Moreover, BDNF synthesis can be both increased and decreased, depending on developmental stage, by GABA_A receptor activation (Marty et al. 1996a). These results suggest a complex interaction between inhibition and BDNF in the hippocampus. Finally, we note that another neurotrophin, NT-3, has been reported to have a disinhibitory effect in somatosensory cortical neurons in culture (Kim et al. 1994), suggesting that neurotrophins may have a more broadly generalized effect on inhibitory activity in the CNS.

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