INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins involved in the regulation of neuron survival and differentiation during development, and it has acute effects on synaptic transmission and plasticity in the adult nervous system (McAllister et al. 1999; Schinder and Poo 2000). Mutant BDNF-deficient mice exhibit deficits in excitatory synaptic transmission, which include alterations in synaptic vesicle docking and synaptic protein distribution (Pozzo-Miller et al. 1999), reduced paired-pulse depression (Asztely et al. 2000) and a decrease in long-term potentiation (Korte et al. 1995; Patterson et al. 1996).

More recently it became clear that BDNF is also a modulator of GABAergic inhibition (Tanaka et al. 1997). However, the currently available results are quite contradictory. In hippocampal slices, synaptic inhibition was enhanced by reduced BDNF expression in bdnf +/− animals (Olofsdotter et al. 2000), and it was depressed by application of exogenous BDNF (Brüning et al. 2001; Frerking et al. 1998; Tanaka et al. 1997). Chronic exposure of hippocampal cultures to BDNF potentiated GABAergic inhibition (Bolton et al. 2000; Vicario-Abejón et al. 1998). In visual cortex slices (Huang et al. 1999) or cerebellar slices (Bao et al. 1999), overexpression of BDNF resulted in an enhancement of GABAergic inhibition. Thus depending on a number of experimental conditions, GABAergic inhibition may either be increased or decreased when the concentration of BDNF in the synaptic environment decreases.

A major point of disagreement is the site of BDNF action. Most studies suggest a presynaptic modulation of transmitter release (Bolton et al. 2000; Frerking et al. 1998; Olofsdotter et al. 2000; Vicario-Abejón et al. 1998), but two groups presented impressive evidence for a postsynaptic depressive effect of BDNF on GABAergic synapses (Brüning et al. 2001; Tanaka et al. 1997).

These discrepancies may be derived from the fact that the systems under study were not equivalent with regard to the maturation of the neurotrophinergic system as well as GABAergic inhibition. In visual structures, the expression of BDNF and its high-affinity receptor TrkB strongly varies with age (Friedman et al. 1991; Klein et al. 1990; Merlio et al. 1992) and neuronal activity (Griesbeck et al. 1999). In addition, GABAergic inhibition undergoes drastic changes during postnatal development (Jüttner et al. 2001). Furthermore, BDNF-dependent myelination (Cellerino et al. 1997) and development of repetitive firing (Rothe et al. 1999) could influence the tests after bdnf inactivation or application of exogenous BDNF.

For our present study on the neurotrophinergic regulation of GABAergic inhibition, we selected the rodent superior colliculus (SC). This part of the brain is particularly rich in GABAergic neurons, and the development of GABAergic synaptic transmission has already been characterized over a wide range of conditions, both in slice preparations (Jüttner et al. 2001) and in cultures (Kirischuk et al. 1999; Kraszewski and Grantyn, 1999).

Postsynaptic Action of BDNF on GABAergic Synaptic Transmission in the Superficial Layers of the Mouse Superior Colliculus

CHRISTIAN HENNEBERGER, RENÉ JÜTTNER, THOMAS ROTHE, AND ROSEMARIE GRANTYN
Developmental Physiology, Johannes Müller Institute of Physiology, Charité, D-10117 Berlin, Germany

Received 13 August 2001; accepted in final form 2 April 2002

Henneberger, Christian, René Jüttner, Thomas Rothe, and Rosemarie Grantyn. Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus. J Neurophysiol 88: 595–603, 2002; 10.1152/jn.00673.2001. The neurotrophin brain-derived neurotrophic factor (BDNF) is involved in numerous aspects of synapse development and plasticity. The present study was aimed at clarifying the significance of endogenous BDNF for the synaptically driven spontaneous network activity and GABAergic inhibition in the superficial layers of the mouse superior colliculus. In this structure neuron survival is unaffected by the absence of BDNF. Two experimental approaches were used: comparison of BDNF-deficient (–/–) and wild-type (+/+ ) mice and blockade of BDNF receptor signaling by the tyrosine kinase inhibitor K-252a. Patch-clamp recordings were performed on horizontal slices during postnatal days 15 and 16. The lack of BDNF in −/− mice caused a significant reduction of the spontaneous action potential frequency and an increase in the pharmacologically induced disinhibition of spike discharge. This change was accompanied by an increase in the amplitudes of GABAergic evoked, spontaneous, and miniature inhibitory postsynaptic currents (IPSCs). BDNF gene inactivation had no effect on the degree of paired-pulse facilitation or the frequency of miniature IPSCs. The increase of IPSC amplitudes by chronic BDNF deprivation was completely mimicked by acute exposure to K-252a in +/+ animals. The enhancement of GABAergic IPSCs in −/− animals was reversed by acute application of 100 ng/ml BDNF, but this rescue was completely prevented by blocking postsynaptic protein kinase C (PKC) activation with the PKC inhibitor peptide 19–31. From these results we conclude that BDNF increases spontaneous network activity by suppressing GABAergic inhibition, the site of action of BDNF is predominantly postsynaptic, BDNF-induced suppression of GABAergic synaptic transmission is caused by acute downregulation of GABA receptors, and BDNF effects are mediated by its TrkB receptor and require PKC activation in the postsynaptic cell.

1992; Warton et al. 1990). Experiments were performed at postnatal day (P) 15. Based on whole cell patch-clamp recordings of inhibitory postsynaptic currents (IPSCs) in horizontal slice preparations from bdnf+/+ and bdnf−/− mice, we intended to answer the following questions: is BDNF involved in the regulation of GABAergic synaptic transmission in the SC? Is the site of action pre- or postsynaptic? Can the effects of BDNF gene inactivation be mimicked by acute block of BDNF receptors? If so, does application of exogenous BDNF reverse the alterations resulting from the chronic absence of BDNF? Does application of exogenous BDNF reverse the alterations resulting from the chronic absence of BDNF receptors? If so, does application of exogenous BDNF reverse the alterations resulting from the chronic absence of BDNF receptors? If so, does application of exogenous BDNF reverse

METH ODS

Animals and slice preparation

Our BDNF knockout mice colony originates from heterozygous mutant mice in a mixed 129Sv, BALB/c, and C57BL/6 background (Ernfors et al. 1994), purchased from Jackson Laboratories (Bar Harbor, ME). These mice displayed the same deficits as the previously characterized BDNF knockout animals generated by Thoenen and colleagues (Korte et al. 1995; Rothe et al. 1999). The bdnf−/− mice are smaller than the wild-type and heterozygous littermates (Korte et al. 1995; Rothe et al. 1999) and die during the third or fourth week after birth. They also develop defects in motor coordination, such as ataxia, spinning during periods of hyperactivity, and recurrent episodes of freezing seizures. Homozygous mice lack detectable levels of BDNF mRNA (data not shown). Homozygous mice are fertile and show no overt abnormalities. All mice were genotyped prior to experiments as described (Henneberger et al. 2001).

If not mentioned otherwise, horizontal slices (150 μm) of the superior colliculus (SC) were prepared from 15- to 16-day-old mice of a previously determined genotype. This postnatal age was chosen to ensure that all animals were in a satisfying functional state, with their eyes already open (eye opening occurs at P13). Although at this age bdnf−/− mice have already a deficit in body weight, SC slices were macroscopically not different. Moreover, cell and synapse counts in the superficial layers of the SC disclosed no differences between +/+ and −/− mice (Henneberger, unpublished result). The day of birth was regarded as P0. Animals were decapitated under ether anesthesia, and the brains were stored in cryoprotective solution (in mM) 120 NaCl, 5 KCl, 10 glucose, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, and 1 MgCl2; the pH was buffered to 7.3 by continuous flow of CO2-95%, O2-5%. In some experiments slices were stored for ≥1.5 h in ACSF containing 200 mM K-252a (Alexis, San Diego, CA). If not stated otherwise, chemicals were purchased from Sigma (St. Louis, MO).

Electrophysiology

Slices were fixed in a submersion recording chamber under a platinum-supported nylon mesh and maintained in ACSF at a constant perfusion rate of 1 ml/min. Slices stored in ACSF containing 200 mM K-252a were also recorded in the presence of 200 mM K-252a. Neurons were approached under direct visual guidance using a ×63 water-immersion objective. In this study, no attempt was made to distinguish between different cell types. When filled with Lucifer yellow, neurons usually had a dendritic field projection of >200 μm and a soma diameter of >12 μm, suggesting that they belong to the class of vertical wide field neurons (Jüttner et al. 2001). However, data of all recordings were pooled despite the uncertainty in neuron classification. Human recombinant BDNF (100 ng/ml; Alomone, Jerusalem, Israel) was dissolved in ACSF containing 0.05% cytochrome C. BDNF was locally applied to the neuron under study using a one-channel superfusion system. Control experiments with cytochrome C demonstrated that the protein application as such had no effect on synaptic transmission. Thus effects observed in the presence of BDNF were not due to unspecified osmotic or mechanical influences. Patch-clamp recordings were carried out at room temperature using an EPC-7 amplifier (List, Darmstadt, Germany). The pipette solution contained (in mM) 120 KCl, 10 EGTA, 10 HEPES, 10 glucose, 1 CaCl2, and 1 MgCl2, and was buffered to pH 7.3. In some experiments, the protein kinase C inhibitor peptide 19–31 (600 nM; Calbiochem, Bad Soden, Germany) was included in the pipette solution.

Action potential activity was recorded with the cell-attached configuration of the patch-clamp technique using the KCl-based internal solution described in the preceding text and voltage-clamp at 0 mV (Fig. 1A). Pharmacological manipulations were done with 200 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX; Tocris, Bristol, UK), 200 μM di-2-amino-5-phosphonovaleric acid (APV), 20 μM t-tubocurarine, 1 μM TTX (Alomone, Jerusalem, Israel), and 50 μM bicuculline methiodiide, respectively.

For whole cell experiments, QX-314 (2 mM; RBI, Natick, MA) was included in the recording electrode to prevent superimposed action potentials. The pipette to bath DC resistance of patch electrodes ranged from 4–6 MΩ (tip diameter 1–2 μm). Series resistance compensation was applied as much as possible (50–70%), only recordings with an series resistance <35 MΩ were accepted. Inhibitory postsynaptic currents (IPSCs) were recorded at a holding voltage (Vh) of −70 mV which resulted in inward IPSCs due to Cl− outflux as a consequence of high intracellular Cl− concentrations. The Cl− equilibrium potential (ECl) was between 0 and −5 mV. GABAergic synaptic transmission was isolated pharmacologically by including 20 μM DNQX, 100 μM APV, and 0.5 μM strychnine in the ACSF. In both genotypes, all the remaining synaptic activity was fully and reversibly blocked by the GABAA receptor antagonist bicuculline (Fig. 2C), indicating that no other afferent type was left after the combined application of the drugs. Spontaneous IPSCs (sIPSCs) were acquired in 1-min recording blocks, ensuring that, throughout the course of an experiment, series resistance did not change significantly. Miniature IPSCs (mIPSCs) were recorded in the same way in the presence of 1 μM TTX to block spike-mediated release. To elicit evoked IPSCs (eIPSCs), neurons or their axons were blindly stimulated through an extracellular glass pipette filled with ACSF (diameter, 2–3 μm, 0.5–2 MΩ). The stimulation pipette was mobile and could be moved across the visual field at a distance of 30–100 μm from the soma to find a presynaptic element in contact with the tested cell. At this stage, no attempt was made to characterize the presynaptic GABAergic neurons or fibers. However, it can safely be assumed that these afferents originate from intrinsic GABAergic interneurons (Jüttner et al. 2001). Pulse duration was 0.5 ms. Stimulus intensities were varied between 2 to 10 μA, a range appropriate to induce unitary responses by minimal stimulation, applying previously described criteria (Jüttner et al. 2001). Basic properties (Table 1) of the recorded neurons did not differ between P15 bdnf+/+ and bdnf−/− mice, indicating that the cells were of the same developmental maturity.

Data analysis

Signals were sampled at a rate of 10 kHz using a 12-bit AD converter and software (WinTida 4.11, HEKA Elektronik, Lambrecht, Germany) and a Bessel filter at 3 kHz. Amplitudes and kinetics of eIPSCs, sIPSCs, and mIPSCs as well as the occurrence of action potentials were analyzed using the in-house-written software PeakCount V2.02 (Henneberger, Berlin), which automatically detects
events as a local minimum of the recorded trace. Decay time constants were estimated by monoexponential fit. Paired-pulse ratio was defined as the ratio of the mean peak amplitudes of the two IPSCs (IPSC2/IPSC1). When the second IPSC occurred on the decaying part of the first, the amplitude of the second IPSC was measured from the initiation point to the peak (relative amplitude). Numerical data are reported as means/SE with n being the number of neurons studied if not stated otherwise. Mann-Whitney U test and Wilcoxon signed-rank test were used for statistical comparisons as appropriate. In figures, significance levels are indicated as *, P < 0.05; **, P < 0.01; ***, P < 0.001, and the numbers of neurons studied in each group are given in brackets.

RESULTS

Inactivation of the BDNF gene decreases the frequency of spontaneous spike discharge in the SC due to an increase in the efficacy of GABAergic inhibition

Spontaneous spike discharge in the superficial layers of the SC was recorded in the cell-attached configuration of the patch clamp technique (Fig. 1A). In bdnf+/+ mice, the spike frequency continuously increased, reaching a maximum at day P20 (Fig. 1B). All the following results were derived from animals at the age P15. Sensitivity to TTX (Fig. 1C) and
DNQX, APV, and d-tubocurarine (Fig. 1D) indicates that the spikes represented Na⁺ action potentials and required glutamatergic and possibly also cholinergic synaptic transmission. The spike frequency increased significantly by addition of bicuculline (Fig. 1E), the difference between control and d-tubocurarine being significant at \( P < 0.05 \). Together, these experiments suggest that the level of spontaneous spike discharge in the superficial SC will depend on the strength of GABAergic inhibition. The phenomenon of pharmacological disinhibition offers an opportunity to identify factors that determine the level of spontaneous network activity in the SC by modulating GABAergic inhibition. The ratio of spike frequency under bicuculline to that in control was higher in \( bdnf^{-/-} \) than in \( bdnf^{+/+} \) animals (Fig. 1F, Table 1). This enhanced disinhibition in \( bdnf^{-/-} \) mice is first evidence that BDNF is one of these factors.

This conclusion is corroborated by the finding that unitary eIPSCs were also larger in \( bdnf^{-/-} \) mice. The amplitude of eIPSCs is the most reliable criterion of synaptic strength. eIPSCs were elicited by minimal electrical stimulation in the presence of DNQX, APV, and strychnine (Fig. 2A). In addition, we recorded spontaneous IPSCs (sIPSCs, not illustrated), which, in part, were generated by spontaneously occurring action potentials in GABAergic neurons. Both e- and sIPSCs were significantly enhanced by the chronic absence of BDNF (Fig. 2B, Table 1). In contrast, no differences were found in the IPSC kinetics (Table 1). These results leave little doubt that BDNF is a major factor in the regulation of GABAergic synaptic transmission in the superficial SC.

A strengthening of GABAergic inhibition can result from a variety of changes, including an increase in the number of synaptic contacts, an increase in the average probability of transmitter release, an upregulation of receptor number or increased activity of postsynaptic receptors. The first possibility cannot be excluded to date. However, counts of GABAergic boutons in thin collicular sections revealed no difference in bouton densities (Henneberger, unpublished result). It therefore seems unlikely that the BDNF-dependent difference in the efficacy of GABAergic synaptic transmission was due to a difference in the number of contacts.

**Paired-pulse facilitation of eIPSCs and frequency of mIPSCs are insensitive to BDNF gene inactivation**

The next possibility is that the IPSC amplitudes increased due to an increase in the probability of transmitter release (\( P \)). An increase in \( P \) is usually associated with a decrease in the degree of paired-pulse facilitation, PPF (Thomson 2000). After eye opening, PPF is the predominant response of GABAergic synapses to paired-pulse activation at short intervals (Fig. 3A). No difference was found in the degree of PPF when comparing \( bdnf^{+/+} \) and \( bdnf^{-/-} \) mice (Fig. 3B, Table 1).

Another way to detect an alteration in \( P \) is to measure the frequency of mIPSCs. However, this parameter, too, was unchanged by the chronic absence of BDNF (Fig. 4B, Table 1). Together, these results suggest that a role of BDNF in presynaptic function is unlikely in these inhibitory synapses.

**BDNF acts at postsynaptic sites**

To further clarify the site of modulatory action of BDNF on GABAergic inhibition in the superficial SC we determined the amplitude of mIPSCs. The amplitude of mIPSCs primarily depends on the number and activity of postsynaptic receptors. mIPSCs were recorded in the presence of TTX, DNQX, APV, and strychnine (Fig. 4A). Figure 4C illustrates that mIPSC amplitudes were indeed larger in animals lacking BDNF. The average amplitudes were 21.3 ± 1.9 pA in \( bdnf^{+/+} \) and 33.7 ± 4.3 pA in \( bdnf^{-/-} \). This difference was significant at \( P < 0.05 \). No difference was found in the rise times and decay time.
TABLE 1. Summary of quantitative data

<table>
<thead>
<tr>
<th></th>
<th>bdnf+/+</th>
<th></th>
<th>bdnf−/−</th>
<th></th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>n</td>
<td>Mean</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>521 ± 101</td>
<td>22</td>
<td>503 ± 91</td>
<td>22</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Whole cell capacitance, pF</td>
<td>16 ± 2</td>
<td>22</td>
<td>16 ± 4</td>
<td>22</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Membrane potential, mV</td>
<td>44.2 ± 2.1</td>
<td>23</td>
<td>44.4 ± 1.5</td>
<td>30</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Spontaneous action potential discharge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Action potential frequency, s⁻¹</td>
<td>5.3 ± 0.6</td>
<td>32</td>
<td>2.5 ± 0.3</td>
<td>21</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Bicuculline to control ratio</td>
<td>1.31 ± 0.17</td>
<td>14</td>
<td>1.99 ± 0.30</td>
<td>11</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>eIPSCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average peak amplitude, pA</td>
<td>93 ± 9</td>
<td>19</td>
<td>178 ± 23</td>
<td>20</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Variation of peak amplitude, CV</td>
<td>0.41 ± 0.03</td>
<td>19</td>
<td>0.40 ± 0.02</td>
<td>20</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Decay time constant τ, ms</td>
<td>14.3 ± 1.2</td>
<td>19</td>
<td>15.8 ± 1.2</td>
<td>20</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>sIPSCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average peak amplitude, pA</td>
<td>33 ± 2</td>
<td>41</td>
<td>46 ± 4</td>
<td>42</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Decay time constant τ, ms</td>
<td>20.4 ± 1.7</td>
<td>21</td>
<td>22.3 ± 1.7</td>
<td>24</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Frequency, s⁻¹</td>
<td>0.88 ± 0.30</td>
<td>21</td>
<td>0.81 ± 0.20</td>
<td>24</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>mIPSCs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average peak amplitude, pA</td>
<td>21.3 ± 1.9</td>
<td>13</td>
<td>33.7 ± 4.3</td>
<td>13</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Time to peak (20–80%), ms</td>
<td>1.0 ± 0.1</td>
<td>13</td>
<td>0.9 ± 0.1</td>
<td>13</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Decay time constant τ, ms</td>
<td>20.4 ± 1.5</td>
<td>13</td>
<td>22.7 ± 2.1</td>
<td>13</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Frequency, s⁻¹</td>
<td>0.11 ± 0.03</td>
<td>13</td>
<td>0.13 ± 0.04</td>
<td>13</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Paired-pulse ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-ms interval</td>
<td>1.37 ± 0.19</td>
<td>18</td>
<td>1.33 ± 0.11</td>
<td>18</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>50-ms interval</td>
<td>1.08 ± 0.11</td>
<td>18</td>
<td>1.17 ± 0.07</td>
<td>18</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>

eIPSC, sIPSC, and mIPSC, evoked, spontaneous, and miniature inhibitory postsynaptic current. constants of mIPSCs. Thus the altered efficacy of GABAergic inhibition must be attributed to a modulatory effect of BDNF at the postsynaptic GABA_A receptor.

**Effect of BDNF gene inactivation on postsynaptic GABA_A receptors and spontaneous network activity in the SC is mimicked by an acute block of TrkB**

Next we wanted to determine the time scale at which GABAergic inhibition and spontaneous spike activity in the SC were influenced by the absence of BDNF. By inducing a pharmacological block of TrkB receptor signaling, one can discriminate between two possibilities: a chronic involvement of BDNF in the formation and activity-dependent reorganization of GABAergic synapses or a more acute modulatory effect of BDNF. To resolve this issue, slices from bdnf+/+ mice were incubated for 1.5 h in 200 nM K-252a, an inhibitor of tyrosine kinase activity (Knüsel and Hefti 1992; Tapley et al. 1992). The following tests were performed in the continuous presence of K-252a. Surprisingly, K-252a completely reproduced the effect of BDNF gene inactivation on the amplitude of mIPSCs without changing their frequency (Fig. 4, B and C).

As K-252a application did not add to the effect of chronic BDNF deprivation in bdnf−/− animals, one has to conclude that long-term BDNF effects and mechanisms compensating the absence of BDNF were apparently not involved. These results are consistent with the idea that the enhancement of GABAergic inhibition in bdnf−/− animals is due to the acute absence of BDNF.

In a number of systems, axon myelination (Cellerino et al. 1997) and spike generation (Rothe et al. 1999) were influenced by the absence of BDNF. It is therefore possible that the difference between chronic BDNF deprivation and TrkB blockade is more conspicuous in the collicular network activity. To answer this question, we determined the frequency of spontaneous spike discharge in the SC from bdnf+/+ and bdnf−/− animals and applied K-252a as described. BDNF gene inactivation resulted in a significant reduction in the frequency of spontaneous spike generation (Fig. 5, Table 1). Again, K-252a not only mimicked the effect of chronic BDNF deprivation, it also added no further effect to the depression already seen in bdnf−/− mice (Fig. 5). Together, these results suggest that the depression of spontaneous network activity in

![FIG. 3. Comparison of the degree of paired-pulse facilitation of evoked IPSCs in bdnf−/− and +/+ mice. A: averaged (n = 30 traces) responses of a bdnf−/− neuron to paired-pulse activation at an interstimulus interval of 25 ms. V_m = −70 mV. Stimulation artifacts are omitted. B: summary of results from bdnf−/− and bdnf+/+ mice.](http://jn.physiology.org/10.2353/jn.2002.88.2.101)

J Neurophysiol • VOL. 88 • AUGUST 2002 • www.jn.org
Exogenous BDNF reverses the effect of gene inactivation on GABAergic synaptic inhibition

This possibility was tested by applying 100 ng/ml BDNF to collicular slices from bdnf−/− animals. We used the following protocol: sIPSCs were recorded for 5 min (control). After this, BDNF was for 10 min locally preapplied via a narrow application pipette. Test records were taken during the last 2 min of BDNF application. This was followed by a 10-min wash period, which again included recording of sIPSCs during the last 2 min. As expected, BDNF had no effect on the frequency and the kinetics of sIPSCs, but the amplitudes of sIPSCs were significantly reduced (Fig. 6A). Average sIPSC amplitudes amounted to 38 ± 3 pA in control, 30 ± 2 pA in BDNF, and 37 ± 3 pA in wash (n = 8; P < 0.01). No change was found in the time constant of decay of sIPSCs. This rescue experiment unequivocally showed that the differences in GABAergic synaptic transmission do not simply reflect developmental consequences of the lack of BDNF but rather reflect the acute requirement for BDNF in ongoing synaptic function as exogenous BDNF modulated GABA<sub>A</sub> receptor function within a few minutes.

Reduction of GABAergic inhibition by exogenous BDNF requires postsynaptic protein kinase C

Protein kinase C (PKC) regulates GABAergic inhibition (Brandon et al. 1999; Filippova et al. 2000), and BDNF may increase the activity of PKC (Tanaka et al. 1997; Zirrgiebel et al. 1995). Therefore activation of PKC may be a necessary step in the intracellular signaling pathway leading to GABA<sub>A</sub>R depression after TrkB activation. To examine this possibility, we applied BDNF to collicular neurons loaded with the PKC inhibitor peptide 19–31 (600 nM). This postsynaptic manipulation completely prevented the depressant effect of exogenous BDNF on sIPSCs (Fig. 6B), confirming the idea that the intracellular signaling pathway following activation of TrkB includes activation of PKC. Thus PKC may play a key role in the regulation of GABA-mediated inhibition in the superficial layers of mouse SC.

DISCUSSION

Comparison of spontaneous spike discharge and IPSC generation in slices from bdnf+/+ and bdnf−/− mice, application

the SC of P15 bdnf−/− mice reflects a relatively narrow alteration—the absence of an acute downregulation of synaptic GABA<sub>A</sub> receptors by BDNF. In this case, one could expect a reduction of GABAergic IPSCs by acute application of BDNF.

**FIG. 4.** Brain-derived neurotrophic factor (BDNF) modulates mIPSC amplitude but not frequency. Recordings were made from superficial SC neurons at V<sub>h</sub> = −70 mV in the presence of 1 μM TTX to prevent action potential generation. A: examples of 200-ms traces of digital records of the holding current and the mIPSCs, obtained from bdnf+/+ (left) and bdnf−/− (right) slices. B: average frequencies of mIPSCs do not differ between bdnf−/− and bdnf+/+ mice, neither with nor without K-252a treatment, which inhibits the TrkB kinase activity. C: average peak amplitudes of mIPSCs are significantly higher in bdnf−/− than in bdnf+/+ mice. This effect can be reproduced in bdnf+/+ slices by exposure to K-252a. In bdnf−/− slices, K-252a treatment has no effect on mIPSC amplitudes. Statistical comparisons are made against the control values (bdnf+/+).

**FIG. 5.** Decreased spontaneous action potential firing in bdnf−/− mice can be attributed to increased inhibition onto superficial SC neurons. Average action potential frequency is significantly lower in bdnf−/− than in bdnf+/+ mice. This deficit can be reproduced in bdnf+/+ slices by exposure to K-252a. In bdnf−/− slices, K-252a treatment has no effect on the action potential frequency.
Acute application of exogenous BDNF reduces sIPSC amplitudes in bdnf−/− mice. Blockade of TrkB receptor activation and blockade of PKC activation allowed us to identify the following mechanisms of BDNF action in the superficial SC. 1) BDNF increases spontaneous network activity by suppressing GABAergic synaptic transmission. 2) The site of BDNF effect is postsynaptic. 3) BDNF causes an acute downregulation of GABA_A receptor-mediated synaptic activity. 4) The depressive effect of BDNF on GABAergic synapses is mediated by its TrkB receptor and requires PKC activation in the postsynaptic cell. Most likely, presynaptic effects and long-term modifications of synaptic structure and function were not involved. Our results extend the list of mechanisms to be considered in further studies on developmental plasticity and activity-dependent map formation in the rodent superior colliculus.

Spontaneous spike generation is a characteristic feature of developing circuits in virtually every part of the nervous system that has been examined to date, including the rodent SC (Itaya et al. 1995). Recently, it was shown by whole cell recording in the cell-attached configuration that spontaneous spike discharge is present in isolated horizontal slices of the mouse SC as early as day P1–3 (Jütten et al. 2001). Here, we demonstrate that the frequency of action potential generation continuously increases from rather low levels at P2 to a maximum at P20. The present study was not aimed at analyzing the developmental changes in ion channel expression which, most likely, underlie or accompany the observed changes in action potential frequency (Jütten et al. 2001; Lo and Mize 1999, 2000; Lo et al. 1998). It was also not intended to identify the origin of spontaneous spike discharge, although our results suggest that glutamatergic synaptic activity is a likely source of spontaneous network activity in this preparation. Interestingly, there was a developmental increase in the frequency of both sEPSCs (Shi et al. 1997; Rothe, unpublished result) and sIPSCs (Jütten et al. 2001). An increase in the firing rate may nonetheless be achieved by a change in the intrinsic firing properties of collicular neurons (Isa and Saito 2001; Lo and Mize 1999, 2000; Lo et al. 1998), a more effective excitation by a given glutamatergic input, or less effective inhibition by a given inhibitory input. Of course, how synaptic afferents affect neuron firing cannot directly be deduced from the amplitude or the frequency of synaptic currents.

The source of BDNF in the superficial layers of the SC is not yet known. In situ hybridization demonstrated mRNA for TrkB in the developing rodent SC (Klein et al. 1990). At the cellular level, TrkB mRNA was homogeneously expressed in most cells of the SC (Merlio et al. 1992; Vizuete et al. 2001). However, BDNF probes labeled only few scattered cells with weak expression of BDNF mRNA during postnatal development (Friedman et al. 1991). It is not excluded that BDNF is delivered to the SC mainly by anterograde transport. In support of this idea, a parallel analysis of BDNF immunoreactivity and in situ hybridization showed many immunoreactive fibers in the superficial SC, but immunoreactive or cRNA-labeled cell bodies were rare (Conner et al. 1997). As rodent retinal ganglion neurons produce BDNF and transport it to the contralateral SC (Caleo et al. 2000), it is conceivable that BDNF of retinal origin activates TrkB in the superficial layers of the SC. However, still very little is known about the cellular localization of TrkB in the rodent SC (Vizuete et al. 2001).

A postsynaptic depressant or potentiating effect of BDNF on GABAergic synapses has already been reported for hippocampal (Brüning et al. 2001; Tanaka et al. 1997) and cerebellar (Bao et al. 1999; Boxall 2000) neurons. The most important open question concerns the nature of the link(s) between TrkB receptors and the synaptic GABA_A receptors. Taking into consideration that counts of inhibitory synapse numbers in the superficial layers of the mouse SC disclosed no differences between +/- and -/- mice (Henneberger, unpublished results), the BDNF–induced downregulation of GABAergic postsynaptic responses can result from at least four different mechanisms: a change in GABA_A subunit composition in the postsynaptic density, increased phosphorylation of GABA_A receptors resulting in decreased single-channel conductance or reduced open probability, decreased local protein synthesis that reduces the rate of postsynaptic receptor insertion, and a change in postsynaptic receptor mobility leading to increased receptor internalization or diffusion to extrasynaptic sites. Unfortunately, none of these mechanisms is sufficiently understood in native GABAergic synapses to unequivocally confirm or reject a role of BDNF at this stage of knowledge.

Can BDNF change the subunit composition in the postsynaptic density of GABAergic synapses?

In cerebellar granule neurons, BDNF enhanced the mRNA expression for the GABA_A receptor subunits α1, α6, and γ2
after 1–2 days in culture (Bullett and Hsieh 2000). In a recessive neurological mutant mouse, stargazer, the failure of cerebellar granule cells to express BDNF resulted in an aberrant GABA_A receptor profile with reduced levels of α6 and β3 subunits (Thompson et al. 1998). So the answer is clearly yes; but we do not yet know whether this mechanism also applies to the SC. In any case, it seems unlikely that the subunit exchange will be complete within 10 min to 1.5 h. This was the time needed to induce or to reverse the postsynaptic depression with exogenous BDNF or with K-252a. We therefore consider this mechanism an unlikely explanation of the present results.

Does phosphorylation of GABA_A receptors account for the reduction of IPSCs?

Neurotrophin-activated tyrosine kinases may gain access to the GABA_A receptor through modification of PKA (Cai et al. 1999) or PKC activity (Papatouk and Reichardt 2001). Phosphorylation of GABA_A receptors may change the opening probability of Cl⁻ channels, the predominant conductance state to which the channels open, or receptor desensitization. However, different kinases may govern GABA_A receptor phosphorylation in different cells. In sympathetic ganglion neurons, PKC-mediated phosphorylation caused a reduction of GABA-activated currents, without a change in their decay kinetics (Krishek et al. 1994). In CA1 pyramidal cells of the hippocampus, mIPSC amplitudes were reduced by PKA activation, but PKC had no effect. In dentate gyrus granule cells, however, PKA was ineffective, but PKC increased the peak amplitude of mIPSCs (Poisbeau et al. 1999).

The present results are consistent with the assumption that BDNF decreases GABAergic IPSCs due to PKC-mediated GABA_A receptor phosphorylation because the pharmacological block of PKC prevented the BDNF-induced depression. However, this mechanism must be regarded as tentative because a BDNF-induced decrease in single-channel conductance or open probability of synaptic Cl⁻ channels has not yet been demonstrated.

Is BDNF involved in local protein synthesis?

It is conceivable that BDNF rapidly regulates the number of GABA_A receptors via local protein synthesis beneath the synapses. In cultured cerebellar granule cells, local up-regulation of synaptophysin and tau proteins by BDNF required ~2 h (Coffey et al. 1997). It remains to be determined whether colocal synaptic proteins are equipped with the essential elements for local synthesis and insertion of transmembrane proteins (Aakalu et al. 2001).

Is postsynaptic receptor internalization or diffusion to extrasynaptic sites regulated by BDNF?

An attractive new possibility to explain the downregulation of GABA_A-R-mediated responses in collicular slices is to assume an alteration in synaptic clustering and/or intracellular receptor trafficking. These mechanisms control the assembly of appropriate subunits into GABA_A receptors and their targeting to synaptic and extrasynaptic membranes (Barnes 2000). Immunocytochemical experiments showed that treatment of hippocampal cultures with BDNF resulted in a decrease of GABA_A receptor subunit immunoreactivity, concurrent with a decrease in the amplitude of GABAergic mIPSCs (Brüning et al. 2001). Moreover, there is already good evidence that the cell surface stability of GABA_A receptors depends on PKC activity (Chapell et al. 1998; Connolly et al. 1999; Filippova et al. 2000). Not only in heterologous expression systems but also in cultured hippocampal and cortical neurons (Brüning et al. 2001; Connolly et al. 1999), GABA_A receptor numbers in the plasma membrane were reduced on PKC activation. PKC-mediated internalization of GABA_A receptors does not involve phosphorylation of any of the known PKC sites of the receptor itself (Connolly et al. 1999; Filippova et al. 2000). It is more likely that the receptor internalization requires PKC-mediated phosphorylation of an unidentified protein. As GABA_A receptors did not degrade after induced internalization but returned to the membrane surface within 24 h (Brüning et al. 2001; Filippova et al. 2000), it was suggested that internalized receptors can exist in an intracellular compartment and can be delivered back to the plasma membrane. Thus by regulating the postsynaptic insertion of GABA_A receptors via PKC activation, BDNF may play a key role in the regulation of GABA-mediated inhibition. Clearly more experiments are necessary to verify this important mechanism.

We thank K. Przedziecki and I. Strömel for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (GRK 238 and SFB 515 B2 to R. Grantyn and RO 925/2-1 to T. Rothe).

REFERENCES


Boxall AR. GABAergic mIPSCs in rat cerebellar Purkinje cells are modulated by TrkB and mGluR1-mediated stimulation of Src. J Physiol (Lond) 524: 677–684, 2000.


ENHANCED GABAERGIC INHIBITION IN BDNF-DEFICIENT MICE


