Effects of BDNF and NT-3 on Development of Ia/Motoneuron Functional Connectivity in Neonatal Rats

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Seebach, Bradley S., Viktor Arvanov, and Lorne M. Mendell. Effects of BDNF and NT-3 on development of Ia/motoneuron functional connectivity in neonatal rats. J. Neurophysiol. 81: 2398–2405, 1999. The effects of neurotrophin administration and neurotrophin removal via administration of tyrosine kinase (trk) immunoadhesins (trk receptor extracellular domains fused with IgG heavy chain) on the development of segmental reflexes were studied in neonatal rats. Brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), trkB-IgG, and trkC-IgG were delivered via subcutaneous injection on days 0, 2, 4, and 6 of postnatal life. Electrophysiological analysis of EPSPs recorded intracellularly in L5 motoneurons in response to stimulation of dorsal root L5 was carried out on postnatal day 8 in the in vitro hemisected spinal cord. Treatment with BDNF resulted in smaller monosynaptic EPSPs with longer latency than those in controls. EPSP amplitude became significantly larger when BDNF was sequestered with trkB-IgG, suggesting that BDNF has a tonic action on the development of this synapse in neonates. Treatment with NT-3 resulted in larger EPSPs, but the decrease noted after administration of trkB-IgG was not significant. Neurotrophins had little effect on the response to high-frequency dorsal root stimulation or on motoneuron properties. Polysynaptic components were exaggerated in BDNF-treated rats and reduced after NT-3 compared with controls. As in control neonates the largest monosynaptic EPSPs in NT-3 and trkB-IgG-treated preparations were observed in motoneurons with relatively large values of rheobase, probably those that are growing the most rapidly. We conclude that supplementary NT-3 and BDNF administered to neonates can influence developing Ia/motoneuron synapses in the spinal cord but with opposite net effects.

INTRODUCTION

The role of neurotrophins during development of the nervous system has been well established. It is now clear that these naturally occurring substances play important roles in the survival and development of sensory and motor neurons (Lindsay 1996). Individual neurotrophins [nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5)] exhibit selectivity for different classes of neurons based on the expression of their corresponding high-affinity tyrosine kinase (trk) receptor (NGF-trkA; BDNF and NT-4/5; trkB; NT-3; trkC) (see reviews in Lewin and Barde 1996; Mendell 1995) although the selectivity is not absolute (Barbacid 1994) (see DISCUSSION). These trk receptors are expressed differentially on developing rat sensory and motoneurons (Mu et al. 1993; Yan et al. 1993).

Recently, it has become clear in the rat that the function of neurotrophins extends beyond the time of birth. NGF plays an important role in the postnatal differentiation and function of nociceptors (see Lewin and Mendell 1993 for review). Administration of exogenous BDNF can improve survival of axotomized motoneurons in neonatal rats (Vejday et al. 1995), and can affect properties of intact motoneurons in the adult (Gonzalez and Collins 1997). NT-4/5 plays an important role in the determination of the conduction velocity of motor axons, and NT-3 influences the conduction velocity of Ia (spindle) afferent fibers and, to a lesser extent, motor axons in adult rats (Munson et al. 1997b). Monosynaptic EPSPs evoked in motoneurons by spindle afferent fibers normally decline in amplitude when the afferent fibers are axotomized (reviewed in Titmus and Faber 1990). This decline is reversed and in fact EPSPs become larger than normal when the axotomized nerve is treated with NT-3 (Mendell et al. 1999; Munson et al. 1997a). The apparent specificity of these actions (NGF: nociceptors; NT-3: spindle afferents and motoneurons; BDNF, NT-4/5: motor axons) is in keeping with the expression of the corresponding trk receptors on subpopulations of sensory neurons and on motoneurons (see reviews in Phillips and Armanini 1996; Sendtner et al. 1996).

The present work was undertaken to explore the role of neurotrophins in the development of the Ia afferent/hindlimb motoneuron system and associated afferent pathways. In a recent paper, the Ia/motoneuron synapse was shown to undergo substantial developmental changes during the first eight postnatal days (Seebach and Mendell 1996a). These changes consisted of increases in motoneuron size (decrease in input resistance, increase in rheobase) and increases in synaptic strength as determined by measurement of EPSP amplitude and susceptibility to synaptic depression during high-frequency stimulation. Also noted were changes in the polysynaptic potentials recorded from the motoneuron after electrical stimulation of the dorsal root (DR) at different ages. BDNF, NT-3, or both may play a role in the specification of motoneuron and afferent-motoneuron synaptic properties during this period, as trkB receptors (activated by BDNF or NT-4/5) are expressed by motoneurons (Mu et al. 1993), and muscle spindle afferents are known to depend on NT-3 for survival (Ernfors et al. 1994; Hory-Lee et al. 1993). The mRNA for each neurotrophin is expressed at maximal levels in skeletal muscle at birth and then declines (Funakoshi et al. 1995; Griesbeck et al. 1995).

We have examined the effect of neurotrophins on the development of this system in two ways. First, we provided exogenous neurotrophins by systemic administration throughout the first neonatal week to see whether the developmental timetable of this system would be affected. Second, we depleted the...
system of endogenous neurotrophins by administering a trk immunoadohesin (Ashkenazi et al. 1993), which, depending on the particular trk immunoadohesin that is used (trkA, trkB, or trkC), binds NGF, BDNF and NT-4 or NT-3, respectively. These immunoadohesins have been shown in vitro (Shelton et al. 1995) and in vivo (Munson et al. 1997b) to block the biological activity of the neurotrophin corresponding to their trk receptor. The results have been reported in abstract form (Seebach and Mendell 1996b) and are compared with observations of the normal maturation of these synaptic and motoneuron properties that were published in a previous report (Seebach and Mendell 1996a).

METHODS

Pregnant Sprague-Dawley rats were monitored at 12-h intervals to establish date and approximate time of birth.

Administration of neurotrophins

Neonatal rats received subcutaneous injections of BDNF, NT-3, trkB immunoadohesin, trkC immunoadohesin, or control vehicle every other day beginning on the day of birth (postnatal day 0, P0). This regimen was employed because daily injections aggrivated the skin in the area of the injection site in some animals. Initial studies were carried out at 2 μg/g based on previous work with NGF in this laboratory (Lewin et al. 1993; Ritter and Mendell 1992). The results at 2 μg/g were very modest and are not included in the results. The data reported here were largely from animals treated with 5 μg/g (in the case of BDNF). 25% of the cells were in animals treated with 10 μg/g with no difference in results from those treated with 5 μg/g. Control animals in the same litter were given like-volume injections of phosphate-buffered saline. Animals receiving either the trkB or trkC immunoadohesin received 10 μg in 50 μl of phosphate-buffered saline every other day from P0 through P6 (dosage suggested by Dr. R. Lindsay, personal communication). The saline-injected control preparations were found to have values of motoneuron and synaptic parameters similar to those obtained previously in untreated preparations (Seebach and Mendell 1996a), and so these groups were amalgamated.

At P8, rats were anesthetized and killed by decapitation. The lumbar region of the spinal cord was removed after ventral laminectomy, hemisected, and placed in a recording chamber perfused with modified Krebs solution as described previously (Seebach and Mendell 1996a; Seebach and Ziskind-Conhaim 1994; Ziskind-Conhaim et al. 1993). Spinal cords were maintained in recording solution at 35°C for ≤12 h.

Measuring motoneuron properties and evoked potentials

Suction electrodes were attached to the L5 dorsal and ventral roots for stimulation. Recordings were made from antidromically identified motoneurons with stable resting membrane potentials more negative than −55 mV using intracellular microelectrodes (80–160 MΩ) filled with 3 M potassium acetate. Motoneuron input resistance and rheobase were measured as described previously (Seebach and Mendell 1996a).

Measurement of responses evoked by dorsal root stimulation

Ten responses to electrical stimulation of the dorsal root (DR), presented at a rate of 0.1 Hz and with an intensity of twice the threshold (2×T) for evoking the monosynaptic potential, were recorded digitally, and then averaged on-line or off-line to estimate EPSP amplitude (Seebach and Mendell 1996a). This stimulation rate did not produce depression of the evoked EPSP at P1–9 (see also FIG. 1. Response of a motoneuron to increasing intensities of dorsal root stimulation at supramaximal group I fiber strength in a BDNF-treated neonatal rat. Note that as stimulus intensity increases (legend gives stimulus intensities for these 3 traces in volts), the polysynaptic components become larger and earlier with no change in the monosynaptic component (*).

Seebach and Ziskind-Conhaim 1994; Ziskind-Conhaim 1988b, 1990). In some cases, this stimulus intensity evoked action potentials either monosynaptically or polysynaptically, and it was necessary to use stimulus intensities as low as 1.5T in those instances. In those cases the maximum amplitude of the monosynaptic and polysynaptic EPSPs probably were underestimated. This would have had a disproportional effect on the findings after treatments that increased EPSP amplitude. Any lowering of stimulus intensity that caused us to underestimate the amplitude of the monosynaptic EPSP also would have caused an underestimate of the magnitude of the polysynaptic EPSP. The fact that we found the largest polysynaptic EPSP components in preparations that received different treatments from those in which monosynaptic components were largest (see RESULTS) suggests that this factor did not influence the reported results to any great extent. Latency was measured from the onset of the stimulus artifact (adjusted to be cathodal stimulation) to the base of the monosynaptic EPSP.

The presence of polysynaptic potentials that often began close to the peak of the monosynaptic potential complicated the measurement of the latter. One approach to overcome this difficulty was to inject depolarizing current through the intracellular electrode to reverse the depolarizing disynaptic IPSP generated in neonatal motoneurons (Seebach and Ziskind-Conhaim 1994). The time of occurrence for the peak monosynaptic response then could be located during off-line analysis and used to measure the amplitude of the peak response in sweeps recorded at resting membrane potential. In addition, in many cases the polysynaptic components of the evoked response were abolished by introduction of a low-Ca2+/high-Mg2+ saline in the bath, which eliminates polysynaptic evoked potentials in this preparation (Pinco and Lev-Tov 1993; Seebach and Mendell 1996a; Seebach and Ziskind-Conhaim 1994) and allowed the confirmation of the latency of the monosynaptic peak. This procedure reduced the amplitude of the monosynaptic potential, but did not affect the latency of its peak (Seebach and Mendell 1996a).

Measurement of polysynaptic responses

In addition to the monosynaptic response, we also determined an index of the polysynaptic response to these stimuli. This was accomplished by measuring the magnitude of the synaptic potential at 10, 20, and 30 ms after the peak of the monosynaptic response. Most of the depolarization induced at these long latencies was produced by higher (electrical) threshold afferents (Fig. 1), and furthermore monosynaptic EPSPs were expected to decay substantially during this time (as seen at low-intensity stimulation or in low-Ca2+ solutions.
Preparation of neurotrophin- and trk immunoadhesin-containing salines

Stock solutions of NT-3, BDNF, trkB-IgG, and trkC-IgG were diluted in phosphate-buffered saline and frozen in 400-μl aliquots. Concentrations were adjusted so that individual injections would range from 25 to 75 μl. Neurotrophins and trk immunoadhesins were received courtesy of Regeneron Pharmaceuticals.

RESULTS

Animal health and weight were monitored carefully. Some animals that received the highest doses of BDNF (10 μg/g) were severely under normal weight by P8, and these animals were excluded from physiological study. The skeletal structure of the low-weight neonates appeared to be fairly normal, but their musculature was slight and they had very little body fat. There were no significant differences in animal weight or observed deviation from normal behavior in the animals used for physiology.

EPSP amplitude

The largest EPSPs in BDNF- and trkB-IgG-treated preparations are compared with the largest EPSP in controls in Fig. 2. Similar comparisons for NT-3 and trkC-IgG are displayed in Fig. 3. After supplementary BDNF the largest EPSP was considerably smaller than in normal controls of the same age, whereas after treatment with the immunoadhesin molecule trkB-IgG, the largest EPSP was considerably larger. Opposite changes also were seen after NT-3 and trkC-IgG treatment where NT-3 increased the amplitude of the largest EPSP and trkC-IgG decreased it. Distributions of EPSP amplitude for each treatment are displayed as cumulative sum histograms in Fig. 4. Note the shift to the left of the control histogram for BDNF and trkC-IgG treatments and a shift to the right for NT-3 and trkB-IgG treatments.

Because EPSP amplitude was measured in controls and after four different treatments with potentially 10 paired comparisons, the Student-Newman-Keuls (SNK) test, which adjusted for multiple comparisons, was carried out. Initially a one-way

\[
\text{depression(\%)} = 100 \times \left(1 - \frac{[(\text{EPSP}_{n+1} + \text{EPSP}_{n})/2]/\text{EPSP}_{0.1 \text{ Hz}})}{1}ight)
\]

This measure is analogous to what previously has been termed facilitation/depression (Collins et al. 1988) and is referred to here as depression (as in Seebach and Mendell 1996a). The amplitude of EPSPs occurring at the end of a burst were adjusted to correct for the sloping baseline produced by previous EPSPs (Collins et al. 1984; Curtis and Eccles 1960). For additional details consult Seebach and Mendell (1996a).

Chemicals and solutions

All solutions were pH 7.2–7.3. The dissecting solution contained (in mM) 140 NaCl, 5.0 KCl, 1.0 MgCl₂, 4.0 CaCl₂, 11 dextrose, and 4.3 HEPES. The recording solution contained (in mM) 116 NaCl, 5.4 KCl, 4.0 CaCl₂, 11 dextrose, 2.0 MgSO₄, 26 NaHCO₃, and 1 NaH₂PO₄. The low-Ca²⁺/high-Mg²⁺ recording solution was modified to contain 0.9 mM Ca²⁺ and 6.0 mM Mg²⁺.
ANOV A on the square root of amplitude, a transformation required to equalize the variance of the amplitude distributions within treatment groups, demonstrated a significant difference in mean amplitude across treatments \( F(4, 105) = 12.2; P = 0.0001 \). The post hoc SNK test (also on \( \sqrt{\text{amplitude}} \)) revealed that EPSP amplitude was depressed significantly \((P < 0.05)\) by BDNF \(2.6 \pm 0.4\) (SE) mV \((n = 20)\) compared with \(5.7 \pm 0.7\) mV \((n = 25)\) in controls and enhanced \((P < 0.05)\) by trkB-IgG \(9.5 \pm 1.4\) mV \((n = 23)\). NT-3 led to a significant increase \((P < 0.05)\) in EPSP amplitude \((10.6 \pm 1.3\) mV; \(n = 23)\), but the decrease in amplitude after trkC-IgG \(3.8 \pm 0.5\) mV; \(n = 19\) \) failed to reach significance (but see DISCUSSION). Amplitude of EPSPs in trkC-IgG-treated preparations was not significantly different from those in BDNF-treated preparations nor was a significant difference observed between NT-3-treated preparations and trkB-IgG-treated preparations.

**EPSP latency**

The changes in monosynaptic EPSP amplitude were accompanied by changes in latency (time from the stimulus artifact to the onset of the EPSP) of the dorsal root-evoked EPSPs. A one-way ANOVA indicated that the five means were not equal \( F(4,105) = 4.6; P < 0.002 \). The post hoc SNK test at the 0.05 level revealed a significant increase in latency of EPSPs in BDNF-treated preparations with respect to the controls \(5.2 \pm 0.2\) ms vs. \(4.5 \pm 0.3\). No significant difference in mean latency was observed comparing any of the other treatments with controls.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Control</th>
<th>BDNF</th>
<th>NT-3</th>
<th>trkB-IgG</th>
<th>trkC-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 Hz</td>
<td>(-95 \pm 1)</td>
<td>(-97 \pm 3)</td>
<td>(-90 \pm 4)</td>
<td>(-92 \pm 1)</td>
<td>(-90 \pm 3)</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 16)</td>
<td>(n = 7)</td>
<td>(n = 13)</td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>33 Hz</td>
<td>(-73 \pm 4)</td>
<td>(-71 \pm 4)</td>
<td>(-70 \pm 9)</td>
<td>(-74 \pm 1)</td>
<td>(-73 \pm 2)</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 16)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 7)</td>
<td></td>
</tr>
<tr>
<td>10 Hz</td>
<td>(-65 \pm 3)</td>
<td>(-72 \pm 2)</td>
<td>(-70 \pm 4)</td>
<td>(-68 \pm 3)</td>
<td>(-69 \pm 4)</td>
</tr>
<tr>
<td>(n = 24)</td>
<td>(n = 17)</td>
<td>(n = 11)</td>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td></td>
</tr>
</tbody>
</table>

Entries are values of average percent synaptic depression (±SD) calculated, as described in METHODS, for a given treatment (columns) and frequency of stimulation (rows). Number of cells is in parentheses. Further details in text.

**Effects on synaptic depression**

Synaptic depression was measured during stimulus trains of 10, 33, and 100 Hz, and the mean values are displayed in Table 1. The mean depression increased progressively as stimulus frequency increased. At each frequency a one-way ANOVA revealed no significant difference in modulation values across treatments \[10\) Hz: \(F(4, 68) = 0.64, P > 0.6; 33\) Hz: \(F(4, 67) = 0.09, P > 0.9; 100\) Hz: \(F(4, 65) = 1.87, P > 0.1\].

**Effects on polysynaptic potentials**

Administration of supplementary BDNF and NT-3 also affected the strength of polysynaptic potentials recorded from the motoneurons after dorsal root stimulation (Fig. 5). To analyze these differences quantitatively, we calculated the difference in amplitude between the potential measured 10 ms after the peak of the monosynaptic response (see METHODS) and the peak of the monosynaptic potential itself (at time 0 in Fig. 5). A one-way ANOVA revealed that there were significant differences in the mean value of this difference over all treatments \(F(4, 103) = 10.3; P < 0.001\). The post hoc SNK test revealed a significant increase in the mean difference in amplitude between the polysynaptic and monosynaptic responses after BDNF compared with the increase in controls \((P < 0.01)\) despite the significant decrease in the amplitude of the monosynaptic EPSP. Similarly, a significant decrease in the polysynaptic potential compared with the monosynaptic peak was observed after NT-3 despite a significant increase in the amplitude of the monosynaptic EPSP \((P < 0.05)\). No significant differences in these measures from control values were seen after trkB-IgG or trkC-IgG treatments despite their effects on the mean amplitude of the monosynaptic EPSP (Fig. 5).

Because the polysynaptic potentials are a composite of excitatory and inhibitory input, the larger polysynaptic component after BDNF treatment could arise from a strengthening of excitatory input and/or a lessening of inhibitory input. Another
amplitude differences among these preparations. Using a multiple regression analysis, we found that the slope of the linear regression for the combined NT-3, trkB-IgG data was 0.28 ± 0.10 (SE), and for the combined BDNF, trkC-IgG data it was 0.02 ± 0.07. The difference between these slopes barely lacked statistical significance ($P = 0.059$) although with this value of probability, it could not be concluded that the slopes were equal. Subsequent linear regression of EPSP amplitude on motoneuron rheobase for the combined NT-3, trkB-IgG data gave a correlation coefficient $r = 0.40$, which is highly significant ($P = 0.01$), indicating preservation of the positive slope of the amplitude versus rheobase relationship after neurotrophin treatments that increased EPSP amplitude. A similar analysis for data from BDNF- and trkC-IgG- treated preparations revealed no significant relationship between EPSP amplitude and rheobase ($r = 0.05; P = 0.79$), indicating that the positive slope of the amplitude versus rheobase relationship is not preserved if EPSP amplitude is diminished.

**DISCUSSION**

BDNF and NT-3 mRNAs are expressed in skeletal muscle at the time of birth, but their levels fall during or soon after the first postnatal week (Funakoshi et al. 1995; Griesbeck et al. 1995). Assuming that levels of NT-3 and BDNF protein also decline during this period, the present results indicate that maintenance of higher than normal levels by provision of supplementary neurotrophins alters the amplitude of the EPSP measured at postnatal day 8. Surplus NT-3 resulted in elevated monosynaptic EPSP amplitude by day 8, whereas BDNF administered in the same dosage and over the same period diminished the mean amplitude of the monosynaptic EPSP.

We also investigated the effects of trkB and trkC immuno-

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**TABLE 2. Mean values of rheobase and input resistance in controls and after neurotrophin treatment**

<table>
<thead>
<tr>
<th></th>
<th>Rheobase, nA</th>
<th>Input Resistance, MΩ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.86 ± 1.61</td>
<td>22.0 ± 15.3</td>
</tr>
<tr>
<td>(25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF</td>
<td>3.68 ± 1.57</td>
<td>16.1 ± 9.0</td>
</tr>
<tr>
<td>(29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-3</td>
<td>2.49 ± 1.66</td>
<td>22.1 ± 17.0</td>
</tr>
<tr>
<td>(23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TrkB-IgG</td>
<td>4.16 ± 1.54*</td>
<td>15.5 ± 4.7</td>
</tr>
<tr>
<td>(27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TrkC-IgG</td>
<td>3.01 ± 1.16</td>
<td>15.1 ± 5.1</td>
</tr>
<tr>
<td>(16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Number of cells in parentheses. * Significantly different from control value ($P < 0.05$). Further details in text.

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**FIG. 6.** Plots of EPSP amplitude vs. motoneuron rheobase for BDNF- and trkB-IgG-treated preparations (top) and NT-3- and trkC-IgG-treated preparations (bottom). Regression lines in each graph fitted to all points on that plot. Further discussion in text.

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possibility is a change in the reversal potential for glycine-and/or GABA$_A$-receptor-mediated potentials, both of which may be depolarizing during development (Serafini et al. 1995; Takahashi et al. 1992). Indeed, in four of four cases with chronic BDNF-treated motoneurons, we could not reverse the composite polysynaptic depolarizing potentials when the cell was depolarized in the range from $-55$ to $-50$ mV, a level at which these potentials are normally reversed (Pinco and Lev-Tov 1993; Seebach and Mendell 1996a; Seebach and Ziskind-Conhaim 1994).

**Effects on motoneuron properties**

EPSP amplitude can be affected strongly by the electrical properties of the postsynaptic cell. To determine whether the changes in EPSP amplitude were in part due to differences in motoneuron development induced by the chronic treatments with BDNF or NT-3 or with their associated trk immunoadhesin, motoneuron input resistance and rheobase were measured. The mean values are presented in Table 2. A one-way ANOVA revealed significant differences in mean rheobase across treatments [$F(4, 115) = 4.74; P = 0.001$]; the post hoc SNK procedure revealed significant differences from controls only after trkB-IgG (Table 2). No significant differences in input resistance across treatments were observed [$F(4, 121) = 2.40; P > 0.05$]. We conclude that the effect of neurotrophins on motoneuron properties is much more modest than their effect on EPSP amplitude in the developing neonatal rat.

**Correlations between amplitude of the monosynaptic EPSP and motoneuron rheobase**

Motoneurons in untreated P8 neonates exhibit a positive correlation between amplitude and rheobase (Seebach and Mendell 1996a) that is reversed from what is observed in the adult rat (Peshori et al. 1998) or in cats (Collins et al. 1988). We investigated whether the treatments that increased EPSP amplitude in these preparations preserved this relationship. To increase power, we combined the data from trkB-IgG-and NT-3-treated preparations and from the BDNF- and trkC-IgG-treated preparations because their mean amplitude did not differ and plotted EPSP amplitude versus motoneuron rheobase (Fig. 6). In carrying out statistical analysis, we transformed amplitude into $\sqrt{amplitude}$ as we had in the analysis of amplitude differences among these preparations. Using a multiple
adhesins, which bind any available BDNF or NT-3 (Ashkenazi et al. 1993), in these preparations. Recent studies indicate that they act in vivo in the rat (Munson et al. 1997b) The effect of the trkB immunoadhesion on synaptic function was highly specific in the sense that its action was opposite to that of BDNF. This suggests that BDNF acts tonically in the postnatal rat to reduce EPSP amplitude (see discussion of potential mechanisms in the following text). TrkC-IgG reduced the mean amplitude of the monosynaptic EPSP (Figs. 2 and 4), which is opposite to the action of NT-3. Although the nominal amplitude of the monosynaptic EPSP (Figs. 2 and 4), which is reduce EPSP amplitude (see discussion of potential mechanisms in the following text). The weaker effect of trkC-IgG compared with that of trkB-IgG has been noted before in vivo. In the adult rat, the effects of trkB-IgG on peripheral axon conduction velocity were more modest than those of trkB-IgG and required a higher dosage (Munson et al. 1997b). Thus the nonsignificant result on EPSP amplitude with trkB-IgG could be explained by the use of too low a dose and should be reexamined with higher concentrations.

We assume IgG is stable under these conditions because of previous findings that IgG injected systemically at days 2–4 still can be detected immunologically in skin and in spinal cord at day 14 (Tonra and Mendell 1997). We also assume that trk receptors are stable because trkB-IgG elicited a significant effect. Because the effects of these 2 trk immunoadhesins are different with respect to synaptic transmission, they are not due to a nonspecific effect of the IgG heavy chain, which is known to penetrate the blood-brain barrier in the neonatal period (Tonra and Mendell 1997).

To interpret these experiments, it is necessary to consider that they are carried out against the backdrop of normal development. Mean amplitude of the monosynaptic EPSP does not change significantly during this period despite substantial growth of the target motoneurons (Seebach and Mendell 1996a). This indicates that the synaptic current produced by the group Ia afferents is increasing throughout this period (to generate EPSPs of the same amplitude). The changes in motoneuron input resistance produced by the various neurotrophin treatments were not significant. Thus they could not account for the 80% increase in the monosynaptic EPSP amplitude after NT-3 treatment, the 67% increase after trkB-IgG, and/or a reduction to 45% of the normal value with BDNF treatment, all of which were significant. We conclude that change in motoneuron input resistance was not the primary cause of the observed changes in monosynaptic EPSP amplitude. Because neither trkB-IgG nor trkC-IgG prevented the decrease in motoneuron input resistance normally observed between postnatal days 2 (41 MHz) and 8 (22 MHz) (Seebach and Mendell 1996a), we also can conclude that at this developmental stage BDNF and NT-3 are not crucial determinants of factors determining input resistance.

If postsynaptic changes are not sufficient to account for the changes in EPSP amplitude, we must consider either changes in the number of afferents or alterations in the synaptic region itself, either presynaptic or postsynaptic. Some evidence for presynaptic changes comes from examination of the latency of the monosynaptic EPSPs. During normal development, EPSP latency after DR stimulation decreases from a mean of 8.5 ms (P2) to a mean of 4.7 ms (P8) (Seebach and Mendell 1996a) as the afferent fibers grow and become myelinated (Friede and Samorajski 1968). In the present experiments, we found that BDNF treatment that reduced EPSP amplitude increased EPSP latency substantially compared with untreated controls. In contrast, both treatments that increased EPSP amplitude (NT-3; trkB-IgG) reduced EPSP latency, although not significantly.

Changes in latency could include changes in afferent conduction time and/or synaptic delay. It was difficult to distinguish between these two sources of latency because the short conduction distance from dorsal root to spinal cord in the neonates prevented us from measuring axonal conduction velocity accurately. Because EPSP latency was increased by BDNF treatment, a decrease in conduction velocity of large muscle afferents might be anticipated in BDNF-treated preparations. Unfortunately, no direct measurements of the effects of BDNF on conduction velocity of sensory afferents appear to be available. However, in the transected peripheral nerve model the other trkB agonist, NT-4/5, does not affect conduction velocity of axotomized sensory afferents in the rat although it does prevent the decline in conduction velocity of transected motor axons (Munson et al. 1997b). Sequestration of trkB agonists with trkB-IgG has no effect on sensory fiber conduction velocity (Munson et al. 1997b). In the cat, NT-4 has little effect on the decrease in conduction velocity of axotomized afferents or axotomized motor axons (Mendell et al. 1999). Thus it remains to be demonstrated whether the present findings can be explained by an effect of BDNF on the development of sensory axon conduction velocity.

When NT-3 is provided to the axotomized MG nerve in adult cats or rats, the anticipated decrease in axonal conduction velocity of large (group Ia) afferents projecting to motoneurons is prevented (Mendell et al. 1999; Munson et al. 1997b). NT-3 also reverses the decline in conduction velocity in diabetic rats (Tomlinson et al. 1996). No significant change in latency after NT-3 treatment was observed under the conditions of these experiments. Whether this is the result of a dosage problem or the inability of NT-3 to affect the conduction velocity of intact afferent fibers is not presently known.

In the adult cat (Collins et al. 1988) or rat (Peshori et al. 1998), there is a negative correlation between EPSP amplitude and motoneuron rheobase, such that motoneurons with large values of rheobase generate small EPSPs on the average, whereas those with small values of rheobase generate large EPSPs. This was not the case in neonatal rats where the correlation between these variables was positive (Seebach and Mendell 1996a). Because rheobase is increasing during development (Seebach and Mendell 1996a), it was suggested that during the first postnatal week, motoneurons growing the fastest, i.e., with the largest values of rheobase, were the ones that had the most highly developed synapses and thus produced the largest EPSPs. Our results indicate that this relationship is preserved if EPSP amplitude is increased but not if it decreases. These data do not presently allow an unequivocal determination of whether the neurotrophin-induced changes in amplitude are confined to motoneurons of a particular range of rheobase. We cannot, of course, be certain that the mechanisms leading to increased EPSP amplitude after NT-3 or trkB-IgG treatment are the same despite the similarity in the outcome.

It is difficult to be more precise about the mechanisms by
which neurotrophins increase or decrease EPSP amplitude. Previous findings indicate a developmental decrease in the susceptibility of these synapses to depression during high-frequency stimulation (Seebach and Mendell 1996a). This is probably due at least in part to the maturation of some aspect of the synapse that gains the ability to recover more completely from the effects of a previous stimulus despite releasing more transmitter. However, myelination of afferent fibers taking place during this period also could contribute by making the impulses more able to be conducted into the fine terminals during high-frequency stimulation. None of the neurotrophin manipulations had a consistent effect on synaptic depression at any frequency of stimulation. This suggests that changes in probability of transmitter release or branch blocking in the terminals, both of which would be expected to change the level of depression, are not responsible for the changes in amplitude observed here. This leaves open the possibility that the changes in EPSP amplitude are the result of altered numbers of transmitter release sites or altered numbers and/or sensitivity of the postsynaptic receptors. Another possibility is a change in the number of spindle afferents, which has been shown to increase in genetically altered mice with muscles that overexpress NT-3 from E11.5 through the first 2 wk of postnatal life (Wright et al. 1997). The number of spindles also is elevated in rats treated with NGF before postnatal day 4 (Miyata et al. 1986). This would indicate a potential role for trkA receptors in this process. Because NT-3 in high doses can activate the trkA receptor (Barbacid 1994), this possibility deserves serious consideration.

The effects of chronic NT-3 administration in increasing the composite EPSP at the Ia-motoneuron synapse is consistent with recent findings in the cat spinal cord where application of NT-3 directly to the axotomized medial gastrocnemius (MG) nerve was found to increase the size of the EPSPs made by the MG afferents on intact LGS motoneurons (Mendell et al. 1999; Munson et al. 1997a). However, the effects of BDNF obtained here are different from those that have been noted in other systems. In the hippocampus (Figurov et al. 1996; Levine et al. 1995; Patterson and Poo 1996) or at the neuromuscular junction (Stoop and Poo 1996), BDNF acts acutely to increase the probability of transmitter release, whereas in the present work with chronic application, the effect was apparently to decrease synaptic efficacy. The present result could be an effect on the timetable of maturation of these synapses during the neonatal period rather than a direct synaptic effect on transmitter release. However, preliminary data indicate that BDNF applied directly to the spinal cord also can depress the monosynaptic EPSP acutely but without the increased response latency (Seebach and Mendell 1996b; unpublished data).

An important caveat in evaluating the differences between this work and previous studies of BDNF action is our inability to confirm that the effects observed after BDNF administration were direct, i.e., not via some other factor induced or upregulated by BDNF. We also cannot be certain that the effects were confined to the Ia-motoneuron synapse. For example, it is likely that trkB receptors are expressed on the presynaptic terminals of at least some of the large diameter afferents (McMahon et al. 1994). If so, BDNF might depolarize these terminals (based on the ability of BDNF to increase probability of release at synapses in the hippocampus or the neuromuscular junction; see preceding text). Thus we can speculate that if periodic administration of supplementary BDNF results in tonic activation of the trkB receptor, it would cause primary afferent depolarization and an indirect decrease in synaptic efficacy of the Ia/motoneuron synapse due to presynaptic inhibition. In addition, because BDNF in larger doses diminished muscle mass (see RESULTS), it is possible that there were subtle retrograde trophic changes in developing the Ia/motoneuron synapse even in cases where animal weight (and muscle mass) was normal (Mendell et al. 1994).

BDNF treatment increased the amplitude of the polysynaptic reflex, which was strikingly different from its effect on the monosynaptic reflex. It is important to note that the polysynaptic reflex differs from the monosynaptic reflex in being produced to a large extent by smaller fibers with a higher electrical threshold than those producing the monosynaptic EPSP (Fig. 1). Thus BDNF may exert different developmental or physiological actions on small diameter afferents, many of which are likely to be cutaneous, than on large muscle afferent fibers. NT-3 also elicited different effects on monosynaptic and polysynaptic potentials, suggesting different actions on large and small afferent fibers. A possible complication in this interpretation is a change in the polysynaptic component’s reversal potential, which after chronic BDNF was at a more depolarized level than in controls (see RESULTS). This would make the depolarizing potential appear larger.

Because the neurotrophins and their antagonists were applied systemically, it is impossible to determine their site of action. The blood brain barrier is not firmly established in these neonatal rats (Tonra and Mendell 1997), and so neurotrophins could act directly on cells in the spinal cord. However, the receptors for these neurotrophins exist on both sensory and motor neurons (McMahon et al. 1994), and it is possible that BDNF and NT-3 are internalized via reaction with their trk receptor in the periphery (or anywhere along the axon) and carried centrally to influence the cell’s metabolism and subsequent growth. The foregoing statement assumes that the interactions are solely on motor neurons and sensory neurons. However, we cannot disregard the possibility that the interactions take place on other neurons and/or glia in the spinal cord.

Although the mechanisms mediating these actions remains obscure for the present, it is clear that the net effects of BDNF and NT-3 administration are relatively straightforward. Whether these effects are restricted to the developmental period is not known. Some recent experiments suggest that BDNF may have different effects on motoneuron properties in the adult rat than those noted here (Gonzalez and Collins 1997), but the effects on synaptic potentials are not known in the adult. From a functional perspective, these results may have important implications for development of segmental reflexes and may provide insights on, or even tools for, how these reflexes might be manipulated in the adult, for example to diminish the monosynaptic reflex during spasticity.

Dr. Nancy Mendell of the Department of Applied Mathematics and Statistics at SUNY-Stony Brook provided help with the statistical analysis. We thank Regeneron Pharmaceuticals, for the generous gifts of BDNF, NT-3, trkB-IgG and trkC-IgG.

This work was supported by National Institute of Neurological Disorders and Stroke Grants RO1 NS-16996 (Javits Neuroscience Award to L. M. Mendell) with additional support from RO1 NS-32264 and PO1 NS-14899.

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