NT-3 Increases Amplitude of EPSPs Produced by Axotomized Group Ia Afferents

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Munson, J. B., R. D. Johnson, and L. M. Mendell. NT-3 increases amplitude of EPSPs produced by axotomized group Ia afferents. J. Neurophysiol. 77: 2209–2212, 1997. We tested the hypothesis that neurotrophin-3 (NT-3) in adult cats can rescue the central synapses made by muscle afferents from the effects of peripheral axotomy. The medial gastrocnemius (MG) muscle nerve in cats was axotomized and capped or axotomized and the distal end provided with either saline or NT-3 by mini-osmotic pump. Four to five weeks later monosynaptic excitatory postsynaptic potentials (EPSPs) elicited by electrical stimulation of the axotomized MG nerve were recorded in intact lateral gastrocnemius/soleus (LGS) motoneurons. The axotomized MG afferents without NT-3 treatment generated EPSPs averaging one-half of the amplitude of those generated by normal intact MG afferents. Axotomized MG afferents treated with NT-3 elicited EPSPs averaging 2.5 times normal amplitude and 5 times the amplitude of those from afferents axotomized but not treated. The very large EPSPs generated by NT-3–treated afferents remained as susceptible to depression during high-frequency stimulation (32 shocks at 167 Hz) as those elicited by untreated axotomized afferents. The arrival of the afferent volley of the cord dorsal potential and the onset of EPSPs were both delayed by axotomy of the group Ia afferents and were both restored by exposure to NT-3. This result suggests that the conduction velocity and thus the caliber of group Ia afferents are also controlled by NT-3. We conclude that the neurotrophin NT-3 has a continuing role in the maintenance of physiological function of muscle afferents in adult mammals.

**INTRODUCTION**

When a peripheral nerve is cut, afferent fibers undergo gradual changes in functional properties. Chief among these changes are a decline in afferent conduction velocity and in the amplitude of the excitatory postsynaptic potentials (EPSPs) they elicit in intact motoneurons (Goldring et al. 1980; Mendell et al. 1995). These changes have been suggested to reflect the loss of trophic factor(s) normally supplied by the periphery (reviewed in Titmus and Faber 1990). Support for this suggestion comes from the fact that axotomized afferents recover after regeneration into either muscle or skin (Johnson et al. 1995; Mendell et al. 1995), either of which might be a source of trophic factors such as neurotrophin-3 (NT-3) (Schechterson and Bothwell 1991).

There are numerous reasons to suppose that NT-3 might serve as such a trophic factor for spindle afferents. The trkC receptor that binds NT-3 is expressed preferentially in the large muscle proprioceptive afferent neurons (McMahon et al. 1994). During development NT-3 is required for survival of large diameter muscle afferents (Hory-Lee et al. 1993) that are absent in animals lacking NT-3 (Ernfors et al. 1994). NT-3 mRNA is expressed in muscle spindles (Copray and Brouwer 1994).

Recently, it has become clear that neurotrophins might act also as a trophic factor in the adult, with NT-3 being specific for promoting the recovery of large diameter afferents after axotomy (Munson et al. 1997) and in experimental peripheral neuropathy (Gao et al. 1995). These associations between NT-3 and development and function of spindle afferent fibers have prompted us to test the effects of applying NT-3 to the cut end of axotomized muscle afferents on the synaptic potentials that they produce in intact motoneurons.

**METHODS**

These data were obtained from 13 adult female cats. Eight of these cats were normal unoperated controls from which data have been published previously (Mendell et al. 1995). In the other five cats (one of these cats also from Mendell et al. 1995; see Table 1) the medial gastrocnemius (MG) nerve was severed in the popliteal fossa, and the MG muscle was excised to prevent self-regeneration. In the cat from the Mendell study, the axotomized nerve was capped with Gore-Tex; in the other four the nerve end was coupled by a Gore-Tex sleeve to a silastic tube and mini-osmotic pump that provided either 0.9% saline (2 cats) or NT-3 at 60 μg/day (2 cats). Acute terminal experiments were performed 4–5 wk after initial surgery.

Acute electrophysiological procedures were as described previously (Collins et al. 1984; Foehring et al. 1986). Conduction time, input resistance, rheobase, and afterhyperpolarization half-decay time (AHP) were determined for antidromically identified, intracellularly recorded lateral gastrocnemius/soleus (LGS) motoneurons with action potential amplitude >60 mV. EPSPs (at 0.5 and 18 Hz and with bursts of 32 shocks at 167 Hz every 2 s, averaged in register) were generated in LGS motoneurons by stimulation of the MG nerve at ~3 times threshold. EPSP modulation during the burst [100×(EPSP30 + EPSP31)/2]/[EPSP1]% expressed the percent increase (positive modulation) or decrease (negative modulation) in amplitude from the first EPSP in the burst to the mean of EPSPs 30 and 31 (Collins et al. 1984).

**RESULTS**

Monosynaptic EPSPs produced in LGS motoneurons by stimulation of MG afferents that were either axotomized or axotomized-and-saline–treated for 4–5 wk averaged 1.0 mV (Table 1). This result represents a decline of 50% from the mean 2.0 mV EPSPs produced by intact MG afferents in intact LGS motoneurons (Mendell et al. 1995). When the cut afferents were treated with NT-3 throughout their 5-wk period of axotomy, EPSP amplitude increased to well in
Table 1. Properties of EPSPs generated by normal, axotomized, and δ-NT-3–treated axotomized MG afferents and of their untreated target LGS motoneurons

<table>
<thead>
<tr>
<th>Synaptic Events</th>
<th>Normal MG afferents</th>
<th>Axotomized MG afferents</th>
<th>NT-3–treated axotomized MG afferents</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPSP amplitude, mV (0.5 Hz)</td>
<td>2.0 ± 1.5</td>
<td>1.0 ± 0.8</td>
<td>5.0 ± 2.6</td>
</tr>
<tr>
<td>Modulation, %</td>
<td>−25 ± 32</td>
<td>−45 ± 18</td>
<td>−47 ± 17</td>
</tr>
<tr>
<td>AHP, ms</td>
<td>31 ± 19</td>
<td>31 ± 18</td>
<td>28 ± 15</td>
</tr>
<tr>
<td>Rheobase, nA</td>
<td>11 ± 7</td>
<td>10 ± 6</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>Input resistance, MO</td>
<td>1.4 ± 0.8</td>
<td>1.5 ± 0.8</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>Motor axon conduction velocity, m/s</td>
<td>86 ± 12</td>
<td>78 ± 12</td>
<td>85 ± 12</td>
</tr>
<tr>
<td>Values are means ± SD. Number of units in parentheses.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 All data in this row from Table 1 of Mendell et al. 1995.</td>
<td></td>
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</tr>
<tr>
<td>2 One 5-wk axotomized cat (12 of the motoneurons in this row) from Mendell et al. 1995; remainder from present experiments (2 saline-treated cats).</td>
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</table>

Excess of normal values, reaching a mean amplitude of 5.0 mV. The very largest EPSPs (>8 mV) were seen in both NT-3–treated cats but not in the eight normal or the three axotomized/un-treated cats (Fig. 1). Figure 1 shows that another effect of NT-3 was to reduce substantially the fraction of EPSPs with amplitude <1 mV. Despite the very large change in EPSP amplitude induced by NT-3, no alteration was observed in EPSP amplitude modulation during high-frequency stimulation (about −45% in both cases; see Table 1). No differences in properties of the untreated target LGS motoneurons were observed, suggesting that motoneuron sampling or altered motoneuron properties did not account for the changes in EPSP amplitude (Table 1).

Untreated axotomized afferents (see also Collins et al. 1986) or those furnished with saline through the cut end exhibited a substantial decrease in conduction velocity. This reduction was evident in the increased latency of the EPSPs generated by the axotomized/un-treated afferents (Fig. 1, inset) and the afferent volley of the cord dorsum potential, which averaged 128% of normal latency (not shown). When the damaged afferents were treated with NT-3 an apparently complete recovery in conduction velocity was observed (Fig. 1, inset and cord dorsum afferent volley) (cf. Munson et al. 1997).

**Discussion**

The amplitude of EPSPs elicited by axotomized afferents in intact motoneurons (Goldring et al. 1980; Mendell et al. 1995) and the conduction velocity of axotomized afferents (Collins et al. 1986) are known to decline as a result of the axotomy. Evidence that such changes could reflect the loss of substances normally retrogradely carried from the periphery was obtained for motoneurons (Czech et al. 1978) and for sympathetic postganglionic fibers treated with blockers of axonal transport (Purves 1976). The present data indicate that exogenous NT-3 applied to axotomized group Ia muscle afferents not only reverses the axotomy-induced decline of conduction velocity but also results in the generating of supernormal EPSPs. This result is consistent with the fact that the trkC receptor that binds NT-3 is found on group I afferents (McMahon et al. 1994). The restored conduction velocity may be due to restoration of axon caliber, resulting from up-regulation of neurofilament production, as shown for nerve growth factor (NGF)-sensitive afferents (Verge et al. 1990).

The present results suggest that NT-3 might serve as a trophic substance mediating synaptic function of group Ia afferent fibers. As stated in the Introduction, this role for NT-3 is not unexpected given its central role in assuring the survival of spindle afferent fibers during development. However, if this explanation is correct it seems clear that the amount and/or effect of NT-3 supplied to the axotomized afferents in these experiments was well beyond normal levels.

How might NT-3 exert control on EPSP size? Remarkably, there was no increase in the susceptibility of these synapses to depression during high-frequency stimulation despite the tremendous increase in EPSP amplitude induced by NT-3. In contrast, when the peripheral nerve is allowed to reinnervate the periphery (Mendell et al. 1995) modulation values become less negative (less abnormal) as EPSP amplitude increases toward normal values. A simple explanation is that NT-3 administration at these levels was much more potent than the peripheral tissue in inducing recovery of the Ia synapse. Transmitter release increased so much (accounting for the very large EPSPs) that the connections remained susceptible to depression in accordance with the normally greater susceptibility of the largest EPSPs to depress during high-frequency stimulation (Collins et al. 1984; Mendell et al. 1995). A second possibility is that the recovery process involved sprouting of terminals of NT-3–treated afferents, with each release site functioning in the same manner as those of untreated axotomized afferents. At the very least we cannot equate the periphery-mediated recovery with the neurotrophin-induced recovery, although these two modes might share certain features.

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FIG. 1. Cumulative sum histograms of amplitudes of excitatory postsynaptic currents (EPSPs) generated in lateral gastrocnemius/soleus (LGS) motoneurons by normal, axotomized-and-saline–treated and axotomized-and-neurotrophin-3 (NT-3)–treated medial gastrocnemius (MG) group Ia afferents. Amplitudes are reduced by axotomy but are made supernormal by NT-3 treatment. Inset: largest EPSPs from each sample. From largest to smallest, EPSPs were generated by NT-3–treated, normal, and axotomized/untreated MG afferents, respectively. Note prolonged latency of EPSP generated by axotomized/untreated afferents and normal latency of EPSP elicited by NT-3–treated MG afferents, indicating effect of NT-3 on conduction velocity of group Ia afferents. Calibrations: 1 mV, 1 ms.

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