Modulation of Motoneuron Excitability by Brain-Derived Neurotrophic Factor

MICHAEL GONZALEZ AND WILLIAM F. COLLINS, III
Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794-5230

Gonzalez, Michael and William F. Collins, III. Modulation of motoneuron excitability by brain-derived neurotrophic factor. J. Neurophysiol. 77: 502–506, 1997. The influence of neurotrophins on motoneuron survival and development has been well documented in cell cultures and neonates. In the present study, the role of brain-derived neurotrophic factor (BDNF) in the maintenance of motoneuron electrical properties was investigated. In adult male rats, BDNF- or saline-saturated gelfoam was inserted between the medial and lateral heads of the gastrocnemius muscles. After 5 days survival, in vivo intracellular recordings were obtained, and motoneuron biophysical properties were measured. In BDNF-treated rats, significant decreases in mean rheobase and in total cell capacitance of medial gastrocnemius motoneurons were observed. In addition, a concomitant increase in input resistance and decrease in membrane time constant were noted in BDNF-treated rats but were not statistically significant. No significant treatment effect was observed in motoneuron conduction velocity, action potential amplitude, equalizing time constant, electrotonic length, afterhyperpolarization amplitude and duration, and membrane potential sag during current injection. The observed changes in motoneuron rheobase and total cell capacitance suggest that application of BDNF produces an increase in motoneuron excitability coincident with a reduction in size. These data are discussed with respect to the possible role of BDNF as a muscle-derived trophic factor for the regulation of motoneuron excitability.

INTRODUCTION

The electrical properties of hindlimb motoneurons vary systematically across motoneurons innervating different muscles as well as those innervating different motor units within the same muscle. Within a population of homonymous motoneurons, rheobase, input resistance, conduction velocity, and size all covary with motoneuron type recruitment order and with motor unit contractile properties (Fleshman et al. 1981; for review, see Binder et al. 1996). In cats, small, low rheobase, high-input resistance triceps surae motoneurons innervate slow-twitch muscle fibers (Type S motoneurons) and are recruited first. Type F motoneurons, which are typically large, high rheobase and low-input resistance motoneurons, innervate fast-twitch muscle fibers and are recruited later. Numerous studies have addressed the question of how this organization is established and maintained, and there is compelling evidence to support the hypothesis that both orthograde and retrograde interactions between motoneurons and the muscle fibers they innervate underlie the differential distribution of motoneuron electrical properties. Specifically, slow muscle fibers appear to exert a retrograde influence on motoneuron electrical properties, whereas Type S motoneurons are able to dictate motor unit contractile speed via an orthodromic mechanism (for review, see Mendell et al. 1994).

Recently, it has been hypothesised that two members of the nerve growth factor family of neurotrophins, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) act as muscle-derived neurotrophic factors for motoneurons (Funakoshi et al. 1993; Koliatsos et al. 1993). Both are produced by skeletal muscle (Funakoshi et al. 1993; Koliatsos et al. 1993) and can be retrogradely transported by motoneurons (Koliatsos et al. 1993; Yan et al. 1993). Further, BDNF has been shown to rescue motoneurons from programmed or injury-induced cell death during development (Oppenheim et al. 1992; Sendtner et al. 1992; Yan et al. 1993). The present study addresses the possible role of muscle-derived BDNF in the regulation of motoneuron electrical properties and is prompted by the observations that BDNF mRNA expression is upregulated in medial gastrocnemius muscle following denervation (Funakoshi et al. 1993; Koliatsos et al. 1993). The results indicate that application of BDNF to the medial gastrocnemius muscle produces a significant increase in electrical excitability of medial gastrocnemius motoneurons. These data have been previously reported in abstract form (Gonzalez and Collins 1995).

METHODS

Data obtained from 94 medial gastrocnemius motoneurons in 18 male Sprague-Dawley rats (350–450 g), anesthetized with a mixture of ketamine/xylazine (90 and 10 mg/kg im, respectively), are included in the present report. The rats were assigned to one of three treatment groups (6 rats/group): BDNF 8 mg/ml, BDNF 16.6 mg/ml, and saline control. The treatments were administered via a 75 mm³ piece of gelfoam (Upjohn) saturated with 50 μl of either BDNF (in phosphate buffered saline, pH 7.0) or saline and inserted into the popliteal fossa of the left leg between the heads of the lateral gastrocnemius and medial gastrocnemius muscles. After 5 days survival, the animals were prepared for in vivo intracellular recording. Each rat was reanesthetized, and the lumbar enlargement of the spinal cord was exposed via a laminectomy from vertebrae T12–L3. The dura was opened and the lumbo-sacral dorsal roots cut bilaterally. The left hindlimb was elevated, the medial gastrocnemius muscle was exposed, and the location of the gelfoam was determined by visual inspection. In two rats, the gelfoam was displaced, and the electromyographical data obtained from those animals are not included in the present report. The medial gastrocnemius nerve was isolated and placed in continuity on bipolar platinum electrodes for stimulation and recording. Fine-tipped glass microelectrodes (10–15 MΩ) filled with 3 M K acetate were used to locate and impale motoneurons. Throughout each experiment, the rat was respirated artificially, and end-tidal CO² was monitored and maintained at 30–35 mm Hg. An
motoneuron in a brain-derived neurotrophic factor (BDNF)-treated (16.6 mg/ml) adult male rat. A: membrane potential response to intrasomal current injection of hyperpolarizing current (−0.5 nA, 100 ms, average of 64 successive sweeps). B: rheobase was determined as minimal amount of intrasomal injected current (0.5–18 nA, 100 ms) needed to generate an action potential. C: intrasomal injection of depolarizing current (10–30 nA, 100 μs, average of 64 successive sweeps) was used to elicit an action potential to measure amplitude and half-decay time of afterhyperpolarization. D: antidromic action potential was elicited by electrical stimulation of medial gastrocnemius nerve (average of 16 successive sweeps).

infrared lamp and heating pad were used to maintain body temperature between 37–38°C. Impaled motoneurons were identified as innervating the medial gastrocnemius muscle by antidromic activation following stimulation of the medial gastrocnemius nerve (Fig. 1D) and by recording the orthodromically conducted response in the medial gastrocnemius nerve after intrasomal stimulation of the neuron (40–70 nA; 100 μs). All data were obtained using an Apple Quadra 650 computer equipped with data acquisition hardware (GW Instruments) and software (WaveMetrics).

Attempts were made to measure the following electrical properties of each motoneuron: input resistance (RN); membrane and equalizing time constants (τm and τe, respectively); membrane potential sag during current injection (Sag); rheobase (Irh); action potential afterhyperpolarization amplitude (AHPamp) and half-decay time (AHP1/2); and amplitude (AP), maximum rate of rise (dV/dt), and conduction velocity (CV) of the antidromic action potential. Motoneuron input resistance was obtained from the change in membrane potential produced by hyperpolarizing current injection (−0.5 nA, 100 ms; Fig. 1A) and was corrected for sag (see Zengel et al. 1985). Motoneuron membrane and equalizing time constants were estimated from the break transient after hyperpolarizing current injection (−0.5 nA, 100 ms) using the peeling method of Rall (1969). Motoneuron rheobase was taken to be the minimal amount of depolarizing current (0.5–18.0 nA, 100 ms) needed to depolarize the motoneuron to threshold (Fig. 1B). Action potential afterhyperpolarization amplitude and half-decay time were measured from the delayed membrane hyperpolarization after the action potential produced by intrasomal current injection (10–40 nA, 100 μs; Fig. 1C). The motoneuron sag and afterhyperpolarization amplitude measurements were normalized by input resistance. Total cell capacitance (Ctot, Eq. 1) and electrotonic length (L, Eq. 2) were calculated using Rall’s equivalent cylinder model equations (Rall 1969; see also Guszafsson and Pinter 1984).

\[ C_{\text{tot}} = \frac{(\tau_m/RN)}{L \tanh L} \]  
\[ L = \frac{\pi}{\sqrt{(\tau_m/\tau_e) - 1}} \]

Only measurements obtained from motoneurons exhibiting action potentials >65 mV and membrane potentials greater than −55 mV were used in our analysis. Data were analyzed using nested analyses of variance and Dunnett’s (1964) post hoc comparisons to control for testing significant treatment effects.

The applicability of Eqs. 1 and 2 depends on the assumption that motoneurons can be approximated as equivalent cylinders with passive membrane of uniform resistivity (Rall 1969). However, morphological and electrophysiological studies of α-motoneurons in the cat suggest that dendritic terminations occur at unequal electrotonic distances from the soma (Barrett and Crill 1974; Clements and Redman 1989; Ulflke and Kellerth 1981) and that membrane resistivity is lower in the soma than in the dendrites (Barrett and Crill 1974; Clements and Redman 1989; Fleshman et al. 1988; Iansek and Redman 1973). Thus the use of Eqs. 1 and 2 can lead to errors in the estimates of τm, as well as L and Ctot (Holmes et al. 1992). Nevertheless, these equations have yielded reasonable estimates of L and Ctot (Gustafsson and Pinter 1984) and are used in the present study to obtain first order approximations of these electrical properties of rat gastrocnemius motoneurons.

**RESULTS**

A summary of electrical properties of medial gastrocnemius motoneurons recorded in saline-treated and BDNF-treated adult male rats is provided in Table 1 and illustrated in Fig. 3. All of the electrical property measurements from motoneurons in saline-treated rats are in close agreement with those obtained by Peshori et al. (K. R. Peshori, W. F. Collins, and L. M. Mendell, unpublished data) under similar conditions but without chronic gel foam inserts. Thus it is unlikely that the gel foam inserts used in the present study produced any significant injury to the medial gastrocnemius nerve or muscle.

It is assumed that most, if not all, of the medial gastrocnemius motoneurons included in the present report are Type F motoneurons. In adult male Sprague-Dawley rats, only 10% of medial gastrocnemius motoneurons are Type S (Gardiner 1993). Action potential afterhyperpolarization has been shown to be the single best indicator of motoneuron type with half-decay times <19 ms being indicative of Type F motoneurons (Gardiner 1993). Also, membrane potential sag during current injection is conspicuously absent in Type S motoneurons; this may account, at least in part, for the longer duration action potential afterhyperpolarization in Type S motoneurons (Gustafsson and Pinter 1985). In the saline treatment group in the present study, 40 of 44 motoneurons had action potential afterhyperpolarization half-decay times <19 ms (mean of 15.4 ms), and 31 of 31 motoneurons exhibited membrane potential sag during current injection (Fig. 1A). These results agree closely with those of Peshori et al. (K. R. Peshori, W. F. Collins, and L. M. Mendell, unpublished results), who, for the same reasons, considered their sample of rat medial gastrocnemius motoneurons to consist primarily of Type F motoneurons.

Application of either 8 or 16.6 mg/ml of BDNF to the medial gastrocnemius muscle produced a significant decrease in the rheobase of medial gastrocnemius motoneurons (35%) compared with the saline-treated controls (Fig. 2A; Table 1). This decrease in motoneuron rheobase appeared to be associated with a dose-dependent increase in motoneuron input resistance and electrotonic length (mean increases of 25 and 11%, respectively) and decrease in membrane time.
TABLE 1. Analysis of BDNF effects on MG motoneuron electrical properties

<table>
<thead>
<tr>
<th></th>
<th>F Saline</th>
<th></th>
<th>BDNF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheobase, nA</td>
<td>7.42</td>
<td>8.07 ± 0.52 (42)</td>
<td>5.25 ± 0.61 (26)*</td>
<td>5.23 ± 0.39 (49)*</td>
<td></td>
</tr>
<tr>
<td>Total cell capacitance, nF</td>
<td>1.51</td>
<td>2.83 ± 0.19 (31)</td>
<td>2.24 ± 0.17 (26)</td>
<td>2.06 ± 0.14 (27)*</td>
<td></td>
</tr>
<tr>
<td>Input resistance, Ω (R_in)</td>
<td>1.14</td>
<td>2.21 ± 0.14 (31)</td>
<td>2.43 ± 0.14 (28)</td>
<td>2.77 ± 0.15 (27)</td>
<td></td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>3.35</td>
<td>4.14 ± 0.20 (31)</td>
<td>3.79 ± 0.23 (26)</td>
<td>3.62 ± 0.13 (27)</td>
<td></td>
</tr>
<tr>
<td>Estimated voltage threshold, mV†</td>
<td>3.05</td>
<td>19.3 ± 1.91 (31)</td>
<td>13.7 ± 1.79 (22)</td>
<td>16.6 ± 1.60 (27)</td>
<td></td>
</tr>
<tr>
<td>Electrotonic length</td>
<td>2.12</td>
<td>1.12 ± 0.03 (31)</td>
<td>1.16 ± 0.05 (26)</td>
<td>1.24 ± 0.04 (27)</td>
<td></td>
</tr>
<tr>
<td>AHP/R_in, mV/Ω</td>
<td>1.15</td>
<td>0.64 ± 0.05 (31)</td>
<td>0.78 ± 0.10 (28)</td>
<td>0.70 ± 0.05 (27)</td>
<td></td>
</tr>
<tr>
<td>AHPms, ms</td>
<td>0.87</td>
<td>15.4 ± 0.71 (44)</td>
<td>13.9 ± 0.34 (37)</td>
<td>15.1 ± 0.46 (47)</td>
<td></td>
</tr>
<tr>
<td>Sagg/R_in, mV/Ω</td>
<td>0.99</td>
<td>0.19 ± 0.01 (31)</td>
<td>0.18 ± 0.01 (27)</td>
<td>0.19 ± 0.01 (27)</td>
<td></td>
</tr>
<tr>
<td>Equalizing time constant (ms)</td>
<td>1.46</td>
<td>0.47 ± 0.03 (31)</td>
<td>0.43 ± 0.03 (26)</td>
<td>0.48 ± 0.02 (27)</td>
<td></td>
</tr>
<tr>
<td>Action potential amplitude, mV</td>
<td>0.87</td>
<td>75.3 ± 1.43 (39)</td>
<td>71.8 ± 1.24 (28)</td>
<td>72.4 ± 0.95 (49)</td>
<td></td>
</tr>
<tr>
<td>Conduction velocity, m/s</td>
<td>1.61</td>
<td>48.8 ± 0.72 (36)</td>
<td>47.8 ± 0.69 (30)</td>
<td>47.7 ± 0.71 (42)</td>
<td></td>
</tr>
</tbody>
</table>

Values in Saline and BDNF are means ± SE with n in parentheses. BDNF, brain-derived neurotrophic factor; MG, medial gastrocnemius. * P < 0.05; Dunnett two-tailed test comparing treatment to saline control. † Estimated voltage threshold = rheobase × input resistance.

The results from the present study demonstrate that application of the trk-B ligand, BDNF, to a muscle can influence electrical and anatomic properties of the motoneurons innervating the muscle. Specifically, reductions in motoneuron rheobase and total cell capacitance were observed, indicating a BDNF-induced increase in motoneuron excitability coupled with a decrease in motoneuron surface area. Of particular constant (mean decrease of 12.5%) (Figs. 2 and 3; Table 1). A decrease in the product of rheobase and input resistance, an estimate of voltage threshold ($V_{thres}$), was also noted (mean decrease of 12%). However, the treatment effects on input resistance, $V_{thres}$, electrotonic length and membrane time constant were not statistically significant (Table 1). No significant treatment effect was observed for motoneuron conduction velocity, action potential amplitude, action potential rate of rise, equalizing time constant, membrane potential sag during current injection or action potential afterhyperpolarization amplitude, and half-decay time (Fig. 3; Table 1).

One possible explanation for the observed decrease in motoneuron rheobase is that medial gastrocnemius motoneurons are smaller in the BDNF-treated rats. To investigate this possibility, an estimate of motoneuron total cell capacitance based upon the measurements of membrane time constant, input resistance and electrotonic length was calculated (see METHODS) and used as an index of total membrane surface area and motoneuron size (Gustafsson and Pinter 1984; Pinter 1990). BDNF produced a dose-dependent decrease in total cell capacitance (Fig. 2) indicative of a decrease in motoneuron surface area and size which, in the case of the 16.6 mg/ml BDNF treatment group, was statistically significant (Fig. 3; Table 1).

DISCUSSION

The results from the present study demonstrate that application of the trk-B ligand, BDNF, to a muscle can influence electrical and anatomic properties of the motoneurons innervating the muscle. Specifically, reductions in motoneuron rheobase and total cell capacitance were observed, indicating a BDNF-induced increase in motoneuron excitability coupled with a decrease in motoneuron surface area. Of particular
lar interest was the lack of significant BDNF-induced changes in other motoneuron electrical properties although a nearly significant increase in input resistance and decreases in membrane time constant and $V_{\text{th}}$ were noted. Based on a passive ohmic model of the neuron, it would be expected that a decrease in motoneuron surface area would result in a decrease in motoneuron rheobase. Thus it is tempting to speculate that the influence of BDNF is primarily a reduction in motoneuron size and indirectly a decrease in rheobase. However, an ohmic model also would predict a significant and corresponding increase in input resistance that was not observed in the present study. Furthermore, the observed decreases in membrane time constant and $V_{\text{th}}$, although not significant, are suggestive of nonohmic BDNF-induced changes in motoneuron membrane properties. Although the present study is limited to examining the effects of BDNF application, it is assumed that the results are indicative of motoneuron trk-B activation and other trk-B ligands, such as NT-4, would be expected to have similar effects. However, additional studies using NT-4 are needed to address this possibility.

A BDNF-induced increase in motoneuron excitability would be expected to have a significant effect on motoneuron function. Motor unit activity is directly related to motoneuron excitability: for a given level of synaptic input, low rheobase motoneurons are activated more readily than high rheobase motoneurons. Thus muscle-derived BDNF could influence motoneuron activity patterns such that motoneurons innervating BDNF-producing muscle fibers would be expected to exhibit a greater level of activity and perhaps be recruited earlier during motor tasks. The functional implications of a possible modulatory influence of BDNF on motoneuron activity and recruitment patterns are supported by recent observations of a differential distribution of BDNF and NT-4 in rat triceps surae muscles. Funakoshi et al. (1995) reported greater NT-4 mRNA expression in the predominantly slow soleus muscle compared with the mixed gastrocnemius muscle and localized NT-4 immunoreactivity to type I myosin-containing muscle fibers in adult rats. We have observed a similar differential distribution of BDNF mRNA expression in rat triceps surae muscle (J. E. Dixon, D. McKinnon, M. Gonzalez, and W. F. Collins, unpublished observations). These observations suggest that muscle-derived BDNF and NT-4 are expressed exclusively by slow muscle fibers.

Based on numerous studies of cat hindlimb motoneurons, it is generally accepted that Type S motoneurons are smaller in size, have lower rheobase values and higher input resistance values than Type F motoneurons (Fleshman et al. 1981) although these differences are less clear in the rat (Bakels and Kernell 1993; Gardiner 1993). Also, many Type F motoneurons convert to Type S phenotype when forced to innervate slow muscles (Dum et al. 1985; Foehring and Munson 1990; Foehring et al. 1987). Thus the observed BDNF-induced decrease in rheobase and total cell capacitance of medial gastrocnemius motoneurons, coupled with the differential expression of NT-4 and BDNF in slow muscle fibers, suggest that the trk-B ligands may be motor unit type-specific trophic links from the muscle fibers to the motoneuron. This may explain the well documented influence of slow muscle on the expression of type S-specific electrical and anatomic properties of motoneurons. However, under the conditions of the present study, BDNF was unable to induce a complete switch from Type F to Type S phenotype: BDNF treatment did not affect motoneuron action potential afterhyperpolarization half-decay time, membrane potential sag during current injection or axonal conduction velocity. Thus it appears that different factors may be involved in the regulation of motoneuron size and rheobase as opposed to action potential afterhyperpolarization and membrane potential sag.

A somewhat surprising result was the lack of an effect of BDNF treatment on motoneuron axonal conduction velocity. On the one hand, this reaffirms our assessment that little or no axonal injury occurred as a result of the chronic gel foam inserts because axonal injury and axotomy produce significant decreases in conduction velocity (Titmus and Faber 1990). However, Munson et al. (1995) recently reported a decrease in gastrocnemius motoneuron axonal conduction velocity in rats treated for 2 wk with local administration of soluble trk-B IgG fusion molecules to the popliteal fossa. Because soluble trk-B IgG is felt to sequester endogenous BDNF and NT-4 (Shelton et al. 1995), thus depriving motoneurons of a muscle-derived source of these neurotrophins, this result suggests that BDNF and/or NT-4 are essential for the maintenance of motoneuron conduction velocity and function. One possible explanation for the lack of a change in motoneuron conduction velocity in the present study is the relatively short BDNF exposure time that was used and that longer exposure times, similar to that employed by Munson et al. (1995), may be necessary to elicit a change in conduction velocity.

In conclusion, the present study demonstrates that application of the neurotrophin BDNF to the gastrocnemius muscle increases motoneuron excitability and decreases motoneuron size as indicated by decreases in rheobase and total cell capacitance, respectively. The lack of a sufficiently large and significant increase in motoneuron input resistance coupled with indications of possible decreases in membrane time constant and voltage threshold suggest that both passive and active motoneuron properties are altered by BDNF treatment. Furthermore, these data indicate a possible functional role of muscle-derived BDNF, or other trk-B ligands, in the trophic influence of slow muscle fibers on motoneuron excitability and size.

We would like to thank W. Hu for expert technical assistance and L. M. Mendell, B. S. Seebach, K. R. Peshori, and D. McKinnon for useful comments on the manuscript. BDNF was generously supplied by Regeneron Pharmaceuticals, Tarrytown, New York.

This work was supported by NIH-9421695 (W. F. Collins) and National Institute of Neurological Disorders and Stroke training grant, 5T32-NS-07371 (M. Gonzalez). Some assistance also was provided by P01-NS-14899 and R01-NS-16996 to L. M. Mendell.

Address reprint requests to W. F. Collins.

Received 14 June 1996; accepted in final form 18 September 1996.

REFERENCES


