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Neuroprotective Effect of Testosterone Treatment on Motoneuron Recruitment Following the Death of Nearby Motoneurons

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Abstract

Motoneuron loss is a significant medical problem, capable of causing severe movement disorders or even death. We have previously shown that motoneuron death induces marked dendritic atrophy in surviving nearby motoneurons. Additionally, in quadriceps motoneurons, this atrophy is accompanied by decreases in motor nerve activity. However, treatment with testosterone partially attenuates changes in both the morphology and activation of quadriceps motoneurons. Testosterone has an even larger neuroprotective effect on the morphology of motoneurons of the spinal nucleus of the bulbocavernosus (SNB), in which testosterone treatment can completely prevent dendritic atrophy. The present experiment was performed to determine whether the greater neuroprotective effect of testosterone on SNB motoneuron morphology was accompanied by a greater neuroprotective effect on motor activation. Right side SNB motoneurons were killed by intramuscular injection of cholera toxin-conjugated saporin in adult male Sprague-Dawley rats. Animals were either given Silastic testosterone implants or left untreated. Four weeks later, left side SNB motor activation was assessed with peripheral nerve recording. The death of right side SNB motoneurons resulted in several changes in the electrophysiological response properties of surviving left side SNB motoneurons, including decreased background activity, increased response latency, increased activity duration, and decreased motoneuron recruitment. Treatment with exogenous testosterone attenuated the increase in activity duration and completely prevented the decrease in motoneuron recruitment. These data provide a functional correlate to the known protective effects of testosterone treatment on the morphology of these motoneurons, and further support a role for testosterone as a therapeutic agent in the injured nervous system.

Keywords

androgen; neuroprotection; morphology; peripheral nerve recording

INTRODUCTION

Motoneuron loss is a significant medical problem and can result from disease processes or from traumatic injuries. For example, amyotrophic lateral sclerosis (ALS) is a fatal disease

characterized by the rapidly progressive loss of motoneurons, affecting four to eight people per 100,000 population per year, with ~5000 new diagnoses made every year in the United States alone (NIH National Library of Medicine, 2008). While ALS is the most prevalent, other diseases including the motor neuron diseases and spinal muscular atrophies are also characterized by progressive loss of motoneurons. Traumatic spinal cord injuries are even more prevalent: in the United States, more than 10,000 people per year survive a spinal cord injury. Of these, around 45% suffer from spinal motoneuron lesions, and this number rises to around 95% for those with lumbar or sacral injuries (Doherty et al., 2002).

We have begun to examine the effects of motoneuron loss on the structure and function of surviving motoneurons using a rat model of motoneuron death. Our previous studies have demonstrated that surviving motoneurons respond to the loss of their neighbors with marked somal and dendritic atrophy (Fargo and Sengelaub, 2004a,b, 2007; Little et al., 2009). Secondary atrophy is responsible for at least some of the movement deficits that accompany degenerative movement disorders and spinal cord trauma. However, motor systems have a high degree of redundancy and display remarkable plasticity in the face of various insults. It has been suggested that this redundancy and plasticity may serve as a substrate for treatment strategies in disease or after spinal cord lesions (Edgerton et al., 2004). Given that we are as yet incapable of replacing lost motoneurons, the best hope for retaining normal function following motoneuron loss may lie in promoting the health of surviving motoneurons by preventing or reversing secondary atrophy.

Gonadal steroids exhibit a wide array of neuroprotective and neurotherapeutic effects (Jones, 1993; Jones et al., 2001; Woolley and Cohen, 2002; Bialek et al., 2004; Tetzlaff et al., 2006; Fargo et al., 2008). For example, after crush axotomy of hamster facial motoneurons, treatment with exogenous testosterone accelerates the rate of both axon regeneration (Kujawa et al., 1991) and recovery of motor function (Kujawa et al., 1989; Kujawa and Jones, 1990). Similar beneficial effects of testosterone treatment have also been shown following axotomy of other spinal and cranial motoneurons (for reviews, see Jones et al., 2001; Fargo et al., 2008). Testosterone also decreases hippocampal neuron death induced by kainate excitotoxicity (Ramsden et al., 2003).

Using our model of motoneuron loss, we have begun to examine the neuroprotective effects of gonadal steroids on the secondary atrophy induced in nearby surviving motoneurons. Our previous experiments have demonstrated that testosterone is indeed neuroprotective following motoneuron loss in both the typical somatic motoneurons innervating the quadriceps muscles and the highly androgen-sensitive, sexually dimorphic motoneurons of the spinal nucleus of the bulbocavernosus (SNB). Treatment with exogenous testosterone prevents or attenuates the dendritic atrophy induced by nearby motoneuron loss, and depending on the timing and nature of the steroid manipulation, can also have beneficial effects on soma size (Fargo and Sengelaub, 2004a,b, 2007; Little et al., 2009). Following motoneuron depletion of quadriceps motoneurons, testosterone treatment attenuates dendritic atrophy in remaining motoneurons, resulting in dendritic lengths that are greater than those of untreated animals by ~65%, and this neuroprotective effect is reflected in electrophysiological measures of motor activation. However, despite testosterone treatment, quadriceps motoneuron dendrites are nonetheless still shorter by almost 40% from those of normal animals, and this is also reflected in their electrophysiological response (Little et al., 2009). In contrast, while the motoneurons of the SNB also respond to the death of nearby motoneurons with dendritic and somal atrophy, testosterone treatment attenuates these regressive changes in morphology to a greater degree than that seen in quadriceps motoneurons. Following motoneuron depletion, testosterone treatment of remaining SNB motoneurons results in dendritic lengths that are greater than those of untreated animals by an average of over 119% and are reduced an average of only ~16% from normal lengths (Fargo and Sengelaub, 2004a,b, 2007). The purpose of the present

experiment was to examine the electrophysiological response properties of SNB motoneurons following the death of nearby motoneurons and, given the larger neuroprotective effect of testosterone treatment seen in SNB morphology, to determine whether treatment with exogenous testosterone would have a greater effect in attenuating potential electrophysiological deficits.

METHODS

Animals

Adult male Sprague-Dawley rats (~100 days old; Harlan, Indianapolis, IN) were maintained on a 12:12 h light/dark cycle, with unlimited access to food and water. We used the toxin saporin, conjugated to the cholera toxin B subunit (Advanced Targeting Systems, San Diego, CA), to kill SNB motoneurons projecting to the right bulbocavernosus (BC) and levator ani (LA) muscles. The BC/LA muscle complex was exposed under ether anesthesia, and the right BC and LA were each injected with 1 μ L of a 0.1% saporin solution. Saporin is a type I ribosome inactivating protein; it kills cells by irreversibly inactivating ribosomes and thereby halting protein synthesis (Stirpe et al., 1983, 1992; Stirpe, 2004). This form of saporin is retrogradely transported from the site of injection, and unilateral injection of saporin into the BC/LA muscle complex kills ~60% of ipsilateral SNB motoneurons within 3 to 6 days, while sparing the nearby contralateral SNB motoneurons (Fargo and Sengelaub, 2004a,b, 2007). Some saporin-injected animals were also castrated and treated with exogenous testosterone via 45-mm long, subcutaneous, interscapular implants of Silastic tubing (3.18 mm outer diameter, 1.57 mm inner diameter) filled with testosterone (4-androsten-17 β -ol-3-one; Steraloids, Newport, RI); such implants produce plasma titers of testosterone steady in the high normal physiological range (Smith et al., 1977) and have previously been demonstrated to prevent or attenuate motoneuron atrophy induced by the death of nearby motoneurons (Fargo and Sengelaub, 2004a,b, 2007; Little et al., 2009). Thus, the three groups in this experiment were untreated normal control animals ($n = 6$), saporin-injected animals ($n = 7$), and saporin-injected animals treated with testosterone ($n = 7$).

Stimulation and Recording

Four weeks after saporin injection and the onset of testosterone treatment, the electrophysiological response properties of the surviving, contralateral SNB were assessed as described by Foster and Sengelaub (2004a). Briefly, animals were anesthetized with chloral hydrate (450 mg/kg body weight, plus incremental doses to maintain areflexia to noxious stimuli) and placed on a 37°C heating pad on a spinal stereotaxic base plate. A high thoracic spinal cord transection was performed to eliminate supraspinal input. The skin over the lumbar spinal column was incised, and the underlying fascia and muscle were dissected to expose the spinous processes of the vertebrae and the proximal portions of the ribs. To reduce potential movement artifacts during stimulation and recording, the spinal column was secured in a vertebral clamp just caudal to the articulation of the final rib. A laminectomy was performed to expose the lumbar spinal cord at the level of the SNB, the dura mater was cut, and the entire region was bathed in warm mineral oil for the duration of the experiment to prevent dessication.

A bipolar hook wire stimulating electrode (model PBCA0750; FHC, Bowdoinham, ME) was placed on left dorsal root L6 (three contiguous dorsal roots carry afferents to the SNB-L5, L6, and S1). The BC/LA muscle complex was exposed and bathed in warm mineral oil, and a recording electrode (model PBCA0750; FHC) was placed on the motor branch of the left pudendal nerve, which contains the axons of BC- and LA-projecting SNB motoneurons. Both the dorsal root and the motor nerve were crushed onto their respective electrodes and severed distal to electrode placement. To prevent any activity in the periphery from introducing artifact into the stimulation pattern, the remaining dorsal roots L5-S1 were severed bilaterally. This

method isolates the central components of the reflex arc (proximal nerves, central synapses, interneurons, and motoneurons) from the peripheral components (distal nerves, dorsal root ganglia, neuromuscular junctions, and muscles) for stimulation and recording.

A computer-based stimulation and recording system (SuperScope II; GWI, Somerville, MA) was used to drive an S48 stimulator (Grass, West Warwick, RI). The stimulus pulse was passed through a constant current unit (model PSIU6E; Grass). To record the actual current delivered during each stimulation, a current probe was attached between the constant current unit and the stimulating electrode. The signal from the recording electrode was passed through a differential AC amplifier (Model 1700; A-M Systems, Carlsborg, WA), filtered (low: 300 Hz; high: 20 kHz), and amplified 1000 $\times$. Signals from both the current probe and the recording electrode were sent to an analog-to-digital acquisition device (InstruNet Model 100B; GWI) and recorded by SuperScope II at an acquisition rate of 10 kHz.

Background activity of the motor nerve was recorded for $\sim$ 13 ms before each stimulus pulse. Stimulus pulses 0.25-ms long were generated once every 15 s, and resultant motor nerve activity was recorded for $\sim$ 87 ms after each stimulus onset. To prevent polarization of the stimulating electrodes, current was temporarily reversed after every 33 or 34 stimulus pulses (with recording suspended). For each animal, the threshold stimulus intensity and the stimulus intensity generating the maximum response were determined empirically. Stimulus intensity was then varied to sample from the entire range of effective stimulus intensities. Approximately 200 traces were generated for each animal. Following the completion of recording, animals were killed by an overdose of urethane and the BC and LA muscles were removed, bisected medially, and weighed.

Data Analysis

Response latency was determined by measuring the delay between the onset of the stimulus pulse at the dorsal root and the beginning of the first spike ("spike" was defined as at least 10 times average background activity) in the motor nerve. It should be noted that this measure includes at least one synaptic delay and is therefore not a measure of conduction velocity. Activity duration was measured by determining the amount of time between the beginning of the first spike and the end of the last spike following a stimulus pulse.

Response magnitude was measured on all traces collected for each animal using the integral of the rectified traces, yielding area under the curve measurements that reflect the activation of all motoneurons during the total duration of the trace. Recruitment curves were then constructed to plot response magnitude as a function of stimulus intensity. Due to individual differences and minor changes in electrode placement, the range of stimulus intensities that produce a motor nerve response varies between animals. To minimize the effects of this variability on statistical analysis, stimulus intensities were standardized across animals. This was accomplished by identifying for each animal the lowest stimulus intensity that produced a maximal response (the "lowest maximally effective stimulus"), then expressing all other stimulus intensities for that animal as a percentage of the lowest maximally effective stimulus.

To minimize the possibility of comparisons being affected by differences in electrode placement or degree of recruitment, average background activity, response latencies, and activity duration were analyzed in traces resulting from stimulus intensities at or above 90% of the lowest maximally effective stimulus for each animal.

Data were analyzed using analysis of variance, either one-way or two-way (one within, one between), as appropriate. *Post hoc* analysis was carried out using Holm-Sidak *t*-tests, with an overall α of $p = 0.05$.

RESULTS

Muscle Mass

Analysis of variance revealed a main effect of treatment group [$F(2, 26) = 32.21, p < 0.001$], a main effect of side [$F(1, 26) = 22.07, p < 0.001$], and an interaction between group and side [$F(2, 26) = 7.81, p < 0.01$]. On the saporin-injected (right) side, saporin reduced muscle mass significantly (429 ± 25.0 mg for saporin-injected animals compared with 770 ± 20.0 mg for normal control animals; $t = 8.05, p < 0.05$; see Fig. 1). Testosterone treatment after saporin injection attenuated this decrease (522 ± 14.9 mg for saporin-injected animals treated with testosterone; compared with untreated saporin-injected animals, $t = 3.30, p < 0.05$) but did not completely prevent it (saporin-injected animals treated with testosterone compared with normal control animals, $t = 5.85, p < 0.05$). Saporin injection also reduced muscle mass on the noninjected (left) side (606 ± 19.8 mg for saporin-injected animals compared with 725 ± 35.0 mg for normal control animals; $t = 2.82, p < 0.05$), but this was prevented by testosterone treatment (702 ± 20.3 mg for saporin-injected animals treated with testosterone; compared with normal control animals, $t = 0.59, ns$; compared with untreated saporin-injected animals, $t = 3.40, p < 0.05$). In normal control animals, there was no difference in muscle mass between the left and right sides ($t = 0.85, ns$). In contrast, muscle mass was reduced on the saporin-injected side in both saporin-injected groups [compared with noninjected (left) side; saporin only group $t = 6.26, p < 0.05$; saporin and testosterone treatment group $t = 6.36, p < 0.05$].

Background Activity

Average background activity differed significantly by group [$F(2, 17) = 6.65, p < 0.01$; see Fig. 2]. Saporin injection reduced average background activity ($2.19 \pm 0.10 \mu V$ for saporin-injected animals compared with $3.33 \pm 0.30 \mu V$ for normal controls; $t = 3.37, p < 0.05$), and treatment with testosterone did not prevent this decrease ($2.40 \pm 0.23 \mu V$ for saporin-injected animals treated with testosterone; compared with normal control animals, $t = 2.84, p < 0.001$).

Evoked Activity

Traces of electrical activity evoked by stimulation of the dorsal root displayed the complex waveform expected from a polysynaptic spinal reflex circuit (Fig. 3).

In normal control animals, activity produced by high intensity stimuli began 2.74 ± 0.34 ms after delivery of the stimulus pulse, on average. However, response latency was significantly lengthened by saporin injection [4.93 ± 0.50 ms for saporin-injected animals; compared with normal control animals, $t = 3.64, p < 0.05$; overall test for the effect of treatment group on response latency $F(2, 12) = 8.90, p < 0.01$; see Fig. 4]. Treatment with testosterone did not attenuate saporin-induced increases in response latency (4.95 ± 0.43 ms for saporin-injected animals treated with testosterone; compared with normal control animals, $t = 3.67, p < 0.05$).

There were also differences in the duration of activity in response to high intensity stimulation [$F(2, 12) = 3.94, p < 0.05$; see Fig. 5]. In normal control animals, activity lasted for an average of 11.66 ± 1.30 ms and never persisted for longer than 17 ms. Saporin injection greatly increased activity duration (30.09 ± 6.97 ms for saporin-injected animals; compared with normal control animals, $t = 2.78, p < 0.05$), and only one saporin-injected animal had an activity duration of less than 17 ms. In saporin-injected animals treated with testosterone, activity duration was intermediate (23.32 ± 3.99 ms): although it was not significantly different from normal control animals ($t = 1.76, ns$), it clearly had more overlap with untreated saporin-injected animals.

Response magnitude was examined across stimulus intensities. As expected, response magnitude increased significantly with stimulus intensity [$F(11, 165) = 36.48, p < 0.001$]. There was also a significant main effect of treatment group [$F(2, 17) = 4.48, p < 0.05$] and a

significant interaction between treatment group and stimulus intensity [$F(22, 165) = 1.83, p < 0.05$; see Fig. 6]. Saporin injection depressed SNB motoneuron recruitment and treatment with testosterone prevented this depression.

DISCUSSION

We have previously shown that testosterone treatment protects motoneurons from atrophy that is induced by the death of nearby motoneurons (Fargo and Sengelaub, 2004a,b, 2007). In this study, we found that this induced atrophy has functional consequences, resulting in concomitant reductions in excitability. Furthermore, similar to its effects on motoneuron morphology, testosterone treatment also protects against reductions in motor activation.

Muscle Mass

Saporin injection decreased BC/LA muscle mass on the injected side of the muscle complex (Fig. 1). This is consistent with the results of previous studies using this model (Fargo and Sengelaub, 2004a,b, 2007). Saporin injection also reduced muscle mass on the noninjected side of the animal as well, although to a lesser extent. This reduction in muscle mass on the noninjected side is not likely due to spread of saporin from the injected side of the muscle as it was not observed in the saporin-injected animals treated with testosterone. Alternatively, consistent with the reduction in motor activation we observed, it is possible that the decreased excitatory input from the moto-neurons resulted in a mild disuse atrophy which was prevented by testosterone treatment.

It is important to consider if the reduction in muscle mass could have influenced the electrophysiological results. For example, it is possible that the reduced muscle mass resulted in decreased trophic support to the innervating motoneurons, which might be expected to influence their electrophysiological characteristics. This seems unlikely, because while muscle mass was restored to normal levels on the noninjected side in the testosterone-treated group, some of the electrophysiological measures (e.g., decreased background activity and increased response latency) were unaffected by testosterone treatment. Furthermore, we have shown in a previous study that steroid treatments can have effects on the electrophysiological properties of this system independent of changes in muscle mass (Foster and Sengelaub, 2004b). In any case, the electrophysiological measurements in the current experiment were taken with the central components of the pathway isolated from the periphery by selective nerve cuts, so the size of the muscles or changes in proprioceptive feedback would not be expected to directly influence these measurements.

Testosterone treatment resulted in higher BC/LA mass on both the noninjected and saporin-injected sides of the muscle. This is an important manipulation check in this experiment, given that testosterone treatment was effective in preventing only some of the observed changes in motor activation following saporin injection. Interestingly, while all of the animals in the study were exposed to testosterone, either endogenously (in the gonadally intact normal and saporin-injected animals) or exogenously (in the saporin-injected castrates treated with testosterone), the protective effects of testosterone were only apparent in saporin-injected animals treated with testosterone implants. In our previous studies, this testosterone manipulation has effectively rescued SNB motoneurons from the induced atrophy seen in gonadally intact, saporin-injected males (Fargo et al., 2004a,b). The superior efficacy of implants over endogenous hormone in supporting androgen-dependent features has been observed previously (e.g., Damassa et al., 1976) and may reflect the difference between a given (and fixed) circulating testosterone level from an implant and the same average (but fluctuating) level of hormone in intact males. Such an effect of implants has been seen in other injury models; for example, after facial nerve crush, nerve regeneration, and recovery of motor function is

accelerated by testosterone treatment, especially that delivered by Silastic implants (Kujuwa et al., 1989).

Background Activity

Average background activity was recorded for ~13 ms before each stimulus pulse was delivered. Background activity was significantly reduced in saporin-injected animals and treatment with testosterone did not prevent this (Fig. 2). This stands in contrast to motoneuron morphology, in which testosterone treatment has been shown to prevent dendritic atrophy under the same experimental conditions (Fargo and Sengelaub, 2004a). This is important for two reasons. First, it indicates that not all of the electrophysiological changes that occur in surviving motoneurons are affected by testosterone treatment. Second, it demonstrates that testosterone treatment in this experiment was not confounded with some procedural variable (such as electrode placement, for example) that would be expected to artificially increase or decrease measures of motor activation. Therefore, any apparently beneficial effect associated with testosterone treatment cannot be explained simply as the result of such a confounding variable.

Evoked Activity

Stimulation of the L6 dorsal root produces activity in the motor branch of the pudendal nerve displaying a complex waveform that is characteristic of polysynaptic spinal reflex circuits (Fig. 3). Several previous studies using a variety of stimulation and recording techniques have also indicated that the BC reflex is mediated by a polysynaptic circuit (Collins, 1985; McKenna and Nadelhaft, 1989; Tanaka and Arnold, 1993; Fargo et al., 2003; Foster and Sengelaub, 2004a).

In intact animals, the first activity spike began slightly less than 3 ms after the stimulus pulse was delivered. In animals in which nearby motoneurons have been killed by saporin injection, response latency rose to nearly 5 ms, and testosterone treatment did not prevent this (Fig. 4). These data suggest that central response latency in this reflex is not controlled by testosterone. This finding is in agreement with a previous report that testosterone has no effect on nerve conduction velocities or central delay in the BC reflex (Tanaka and Arnold, 1993). This stands in contrast to the effect of gonadal steroids on response latency in the peripheral motor components of the SNB system. When SNB motor axons are stimulated in the periphery and EMG is recorded at the BC muscle, response latency is increased by castration and treatment with either estradiol or dihydrotestosterone prevents this increase (Fargo et al., 2003; Foster and Sengelaub, 2004b). Additionally, the present results contrast with the effect of testosterone on dendritic morphology, as testosterone treatment attenuates the dendritic atrophy seen in surviving motoneurons after saporin injection (Fargo and Sengelaub, 2004a,b,2007). Thus, the response latency data presented here are an example of a change in the electrophysiological response properties of the SNB system that is (a) independent of dendritic morphology, at least to some degree and (b) apparently not amenable to testosterone treatment.

The duration of activity was defined as the amount of time between the beginning of the first activity spike and the end of the last activity spike. In animals in which nearby motoneurons have been killed by saporin injection, activity duration increases significantly, and testosterone does not effectively prevent this (Fig. 5). As with response latency, activity duration appears to change independently of dendritic morphology and to not be amenable to testosterone treatment. In normal males, SNB motoneurons produce highly synchronized activity that is punctuated by short interruptions during which the motoneurons are presumably refractory to continuing input (Holmes et al., 1991; Holmes and Sachs, 1994). Perhaps the remaining motoneurons in the saporin-injected groups no longer all fire together, allowing later input to produce motor activation and thereby increasing activity duration. SNB motoneurons are connected to one another by gap junctions, and gap junction-mediated dye coupling is apparent

both ipsilaterally and across the midline with contralateral SNB motoneurons (Coleman and Sengelaub, 2002). The electrophysiological response properties of SNB motoneurons are altered by pharmacological blockade of gap junctions (Foster and Sengelaub, 2004a), and it is possible that synchronized activity in the SNB is mediated in part by gap junction coupling. Thus, it is possible that in the saporin-injected groups, the loss of the contralateral motoneurons which would normally be connected via gap junctions to the remaining motoneurons on the noninjected side leads to a loss of synchronous firing. Another possible explanation for the increase in activity duration is related to the fact that the polysynaptic input to SNB motoneurons is mixed, producing both EPSPs and IPSPs (Collins, 1985). It is possible that the death of nearby motoneurons results in reorganization of inputs into the surviving motoneurons that favors EPSPs over IPSPs.

The recruitment curve plotting response magnitude against stimulus intensity reveals that SNB recruitment is depressed in animals in which nearby motoneurons have been killed by saporin injection, and testosterone treatment prevents this depression (Fig. 6). This result means that the overall excitability of the motor pool as a whole is decreased by the death of nearby motoneurons and that testosterone treatment allows the motor pool to maintain a normal level of excitability. This result maps directly onto dendritic morphology in these groups. In untreated saporin-injected animals, dendrites shrink by over 50%, and this would be expected to decrease the number of synapses made onto each individual moto-neuron, thus decreasing the likelihood that it would produce an action potential in response to any given stimulus delivered to the dorsal root. Testosterone treatment greatly reduces the secondary dendritic atrophy, thus presumably maintaining a normal level of synaptic input, and therefore a normal level of motor pool excitability. In addition to providing surface area for synaptic input, motoneuron dendrites also mediate a persistent inward current that amplifies the input–output gain by up to several fold (Heckmann et al., 2005, 2008). This current provides another potential explanation for the apparent relationship between dendritic length and motor excitability in this experimental model. Reduced input–output gain in motoneurons has also been observed in carpal tunnel syndrome, even in cases in which electrodiagnostic testing indicates normal motor nerve conduction velocities (Ginanneschi et al., 2006).

It might seem like a paradox that the death of nearby motoneurons would both increase activity duration and decrease motoneuron recruitment. However, activity duration and recruitment are fundamentally different ways of examining motor activation. Activity duration takes into account only the length of time between the beginning of the first activity spike and the end of the last activity spike and does not take into account the magnitude of the motor response. Therefore, a longer duration of activity can be produced by a smaller number of responding motoneurons. Additionally, activity duration was measured only during high intensity stimulations. Recruitment, on the other hand, is by definition measured over a range of stimulus intensities and is a measure of overall response magnitude rather than duration.

According to the Henneman size principle, moto-neurons tend to be activated in a size-dependent order, with smaller motoneurons being recruited before larger motoneurons (Henneman et al., 1965a,b). Although we did not directly measure the relationship between motoneuron size and recruitment order in this experiment, we saw no frank evidence of any changes in the size-recruitment relationship in the saporin-injected groups. In fact, the recruitment curves produced by each group appeared grossly similar with the exception of decreased slope in the untreated saporin-injected group. This was not surprising, as several authors have noted that the size principle is capable of reasserting itself after injury (Cope and Clark, 1993; Gordon et al., 2004), even in experimental preparations that reduce overall motor output (González-Forero et al., 2004).

Functional Correlate of Testosterone's Protective Effect on Anatomy

A clear pattern emerged in the data from this experiment: the death of nearby motoneurons led to changes in several measures of central SNB electrophysiology, some of which were not prevented by testosterone treatment. The death of nearby motoneurons depresses average background activity and increases both response latency and activity duration, and testosterone treatment has no effect on these measures. This pattern is different from that seen in the morphology of SNB dendrites, in which testosterone treatment greatly reduces the profound atrophy secondary to the death of nearby motoneurons. Therefore, there is no simple relationship between these measures of SNB motor function and testosterone's protective effect on SNB morphology.

Of course, it is unlikely that shrunken somata and dendritic arbors are the only neuroanatomical differences between normal controls and animals in which nearby motoneurons have been killed by saporin injection. These regressive changes are likely to result in the reorganization of other inputs into the surviving SNB motoneurons, such as those coming from interneurons. This hypothetical reorganization of the spinal reflex circuitry would be expected to give rise to changes to the electrophysiological response properties of the reflex, such as the changes in background activity, response latency, and activity duration reported here. In fact, in the only other reports in the literature concerning the electrophysiology of surviving motoneurons in close proximity to an injury site at which motoneurons have been killed, the authors note changes in reflex properties that appear to be caused by just such spinal reorganization (Holmberg and Kellerth, 1996; Holmberg and Kellerth, 2000). Some of these hypothetical changes in anatomy of the reflex pathway may not respond to testosterone treatment, and the electrophysiological changes arising from them would therefore also be expected to be unresponsive to testosterone treatment.

One measure of SNB electrophysiology, however, does appear to correlate with SNB dendritic morphology following the death of nearby motoneurons: SNB recruitment is depressed following the death of nearby motoneurons, but testosterone treatment prevents this depression. This pattern of results exactly mirrors the protective effect of testosterone on the dendrites of SNB motoneurons and suggests that recruitment may be mediated in part by motoneuron dendritic length, perhaps by the increased surface area of larger dendritic arbors providing sites for additional afferent input.

A similar pattern of results has been found in the motoneurons innervating the vastus lateralis of the quadriceps (Little et al., 2009). When the nearby vastus medialis-projecting motoneurons are killed by saporin injection, vastus lateralis motoneurons respond with both profound dendritic atrophy and a marked decrease in motor excitability. Testosterone treatment is neuroprotective in attenuating both of these regressive changes. However, in quadriceps motoneurons, the attenuation is only partial, whereas this study and previous studies (Fargo and Sengelaub, 2004a,b, 2007) reveal a greater protection of both dendritic morphology and motoneuron recruitment in SNB motoneurons. A likely explanation for this difference lies in the differential expression of androgen receptors in these two systems (Little et al., 2009). SNB motoneurons abundantly express androgen receptors, and almost 70% of SNB motoneurons are labeled at 5 $\times$ background; in contrast, less than 3% reach this criterion in other motoneuron populations (Breedlove and Arnold, 1980, 1983). Using steroid autoradiography, we have found that SNB motoneurons accumulate testosterone at almost three times the density seen in quadriceps motoneurons (Little et al., 2009). Similarly, the SNB target muscles in males are enriched for androgen binding sites and androgen receptor protein compared with other striated muscles (Dubé et al., 1976; Tremblay et al., 1977; Monks et al., 2006). Androgen receptor protein is present in substantially higher concentrations in the levator ani compared with other skeletal muscle (Monks et al., 2006), and the bulbocavernosus and levator ani muscles have over four times as many binding sites for testosterone than are present in the quadriceps muscles.

(Dubé et al., 1976). Thus, as differences in the density of androgen receptors are thought to underlie differences in androgen responsiveness across tissues (Monks et al., 2006), the higher density of androgen receptors in the SNB system might result in a greater protective effect of testosterone.

CONCLUSIONS

The purpose of this experiment was to determine whether any electrophysiological changes accompany SNB motoneuron atrophy induced by the death of nearby motoneurons, and to determine whether treatment with exogenous testosterone would prevent these changes. Several measures of SNB electrophysiology were affected by the death of nearby motoneurons: average background activity, response latency, activity duration, and recruitment. Testosterone treatment restored recruitment to normal levels. These data indicate that the secondary atrophy of SNB motoneurons after the death of nearby motoneurons is indeed accompanied by changes in the functional electrophysiology of the nucleus. More importantly, they demonstrate that testosterone treatment, in addition to restoring normal dendritic morphology, restores the ability of the system to produce a normal motor recruitment pattern.

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Figure 1.

BC/LA muscle masses for normal control animals (Normal), saporin-injected animals (SAP), and saporin-injected animals treated with testosterone (SAP+T) for both the right (open bars) and left (filled bars) sides. In saporin-injected groups, injections were made into the right BC/LA muscles. Consistent with previous studies with this model, saporin injection reduced BC/LA muscle mass. Testosterone treatment increased BC/LA muscle mass on both the noninjected and saporin-injected sides. See the text for details of statistical analysis. Bar heights represent means $\pm$ SEM. * indicates significantly different from left side, within group ($p < 0.05$). † indicates significantly different from untreated saporin-injected animals, within side ($p < 0.05$).

Figure 2.

Average background activity levels for normal control animals (Normal), saporin-injected animals (SAP), and saporin-injected animals treated with testosterone (SAP+T). Background activity was significantly depressed in saporin-injected animals, and treatment with testosterone did not prevent this. Bar heights represent means $\pm$ SEM. * indicates significantly different from normal control animals ($p < 0.01$).

Figure 3. Representative rectified traces from a normal control animal (Normal), a saporin-injected animal (SAP), and a saporin-injected animal treated with testosterone (SAP+T).

Figure 4.

Average response latencies for normal control animals (Normal), saporin-injected animals (SAP), and saporin-injected animals treated with testosterone (SAP+T). Response latencies were significantly increased in saporin-injected animals and treatment with testosterone did not prevent this. Bar heights represent means $\pm$ SEM. * indicates significantly different from normal control animals ($p < 0.05$).

Figure 5.

Average activity durations for normal control animals (Normal), saporin-injected animals (SAP), and saporin-injected animals treated with testosterone (SAP+T). Activity duration was significantly increased in saporin-injected animals. Although saporin-injected animals treated with testosterone did not differ significantly from either normal control animals or untreated saporin-injected animals, they were much more similar to untreated saporin-injected animals. Bar heights represent means $\pm$ SEM. * indicates significantly different from normal control animals ($p < 0.05$).

Figure 6.

Recruitment curves for normal control animals (filled circles), saporin-injected animals (open circles), and saporin-injected animals treated with testosterone (triangles). Stimulus intensity was defined within each animal as a percentage of the stimulus pulse that produced the largest response in that animal. Response magnitude is measured as average area under the curve in rectified traces. Recruitment was significantly depressed in saporin-injected animals, but treatment with testosterone prevented this. See the text for details of statistical analysis. Marker heights represent means $\pm$ SEM.