Nerve Growth Factor Decreases Potassium Currents and Alters Repetitive Firing in Rat Sympathetic Neurons

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Luther, Jason A. and Susan J. Birren. Nerve growth factor decreases potassium currents and alters repetitive firing in rat sympathetic neurons. J Neurophysiol 96: 946–958, 2006. First published May 17, 2006; doi:10.1152/jn.01078.2005. The sympathetic nervous system is an essential regulator of cardiovascular system and interactions with target tissue regulate sympathetic neuronal properties. The heart produces nerve growth factor (NGF), which promotes sympathetic noradrenergic innervation of cardiac tissue and affects sympathetic synaptic strength. Neurotrophins, including NGF, are important modulators of synaptic plasticity and membrane electrical properties. Here we show that acute application of NGF causes a change in the repetitive firing pattern of cultured sympathetic neurons of the rat superior cervical ganglion. Neurons fire fewer action potentials in NGF, but with increased frequency, demonstrating an NGF-dependent change from a tonic to a phasic firing pattern. Additionally, NGF decreases the spike time variance, making spikes more tightly time locked to stimulus onset. NGF causes a decrease in the amplitude of both calcium-dependent and independent potassium currents, and inhibition of calcium-dependent potassium currents using CdCl2 reproduces some, but not all, of the firing properties induced by NGF. This study suggests that NGF release from cardiac tissue may act to modulate the repetitive firing properties of sympathetic neurons to tune their output to meet the physiological needs of the organism.

INTRODUCTION

The sympathetic nervous system is essential for homeostatic regulation of many critical physiological processes (Loewy 1990). Interaction with target tissues has a profound influence on morphological and electrophysiological properties of sympathetic neurons, as well as regulating transmitter and neuropeptide content (Anderson et al. 2001; Andrews et al. 1996; Kukula and Szulczyk 1998; Raucher and Dryer 1995; Szulczyk et al. 2006; Voyvodic 1989). Many of these properties differ between classes of sympathetic neurons projecting to distinct targets, presumably reflecting their disparate physiological roles and demonstrating target-specific regulation of neuronal properties (Landis 1990).

Nerve growth factor (NGF) signaling from the heart is an important regulator of sympathetic synaptic activity. Developmentally, NGF secreted by cardiac myocytes promotes survival and positively regulates the arborization of sympathetic neurons and the density of sympathetic innervation to the heart (Hassankhani et al. 1995; Ieda et al. 2004; Korsching and Thoenen 1983). Previous work showed that NGF increases noradrenergic synaptic contacts between cocultured superior cervical ganglion (SCG) neurons and cardiac myocytes (Lockhart et al. 2000). Additionally, NGF rapidly increases noradrenergic synaptic strength in myocyte—sympathetic neuron co-cultures through a presynaptic mechanism dependent on the NGF high-affinity tyrosine kinase receptor, TrkA (Lockhart et al. 1997). Although rapid synaptic changes are driven by neurotrophins such as NGF, little is known about the underlying cellular regulation supporting these changes.

One way that neurotrophins may act to modulate presynaptic function is to alter the membrane electrical properties controlling firing pattern and calcium entry at the synapse. Neurotrophins, including NGF, regulate neuronal ion channels and membrane electrical properties in both central and peripheral neurons (Adamson et al. 2002; Baldelli et al. 2000; Holm et al. 1997; Levine et al. 1995; Zhang et al. 2002). For example, NGF increases the amplitude of calcium-dependent potassium currents in cultured chick sympathetic neurons (Raucher and Dryer 1995) and regulates both calcium and sodium currents in cultured frog sympathetic ganglion B cells (Lei et al. 1997, 2001). Immunization of rats with NGF leads to a decrease in systemic NGF and a decrease in the amplitude of the tetrodotoxin-resistant sodium current in dorsal root ganglion neurons, suggesting that NGF positively regulates that current in vivo (Fjell et al. 1999).

In addition to these long-term changes in ionic currents NGF also influences neuronal membrane electrical and firing properties on relatively short timescales. NGF treatment increases L-type calcium current within minutes in cultured PC12 cells (Jia et al. 1999) and rapidly alters the firing properties of dorsal root ganglia sensory neurons by increasing sodium and decreasing potassium current amplitudes (Zhang et al. 2002). These studies suggest that target-derived neurotrophin signaling could be an important mechanism for the rapid regulation of membrane electrical properties in sympathetic neurons as well.

Release of NGF from target tissue is physiologically regulated and may represent an important feedback mechanism controlling sympathetic output. NGF protein and mRNA are reduced in cardiac myocytes in heart failure models and plasma NGF concentrations are reduced in human heart failure patients (Kaye et al. 2000; Qin et al. 2002). NGF release from the heart is altered by ischemia, heart failure, and intracardiac norepinephrine infusion (Hiltunen et al. 2001; Qin et al. 2002) and is regulated by estrogen and progesterone (Bjorling et al. 2002; Kaye et al. 2000). Norepinephrine content and TrkA protein is downregulated in sympathetic synaptic terminals in cardiac tissue during congestive heart failure (Kaye et al. 2000; Qin et al. 2002; Qin et al. 2006). Nerve growth factor decreases potassium currents and alters repetitive firing in rat sympathetic neurons.
NGF alters potassium currents and repetitive firing

al. 2002). Thus target-derived NGF signaling may convey information back to sympathetic neurons concerning the physiological state of target systems.

NGF signaling from the heart appears to be important in regulating the morphological and synaptic properties of sympathetic neurons both developmentally and in the adult, although the mechanisms underlying acute effects of NGF on these neurons are not well characterized. Here we present evidence that short-term exposure to NGF rapidly alters potassium currents and affects neuronal firing patterns of rat SCG sympathetic neurons in vitro. These data suggest that NGF signaling from heart cells provides a target-specific mechanism for regulating the repetitive firing characteristics of sympathetic neurons. Disregulation of NGF signaling may lead to abnormal sympathetic output and contribute to cardiovascular diseases such as congestive heart failure and hypertension.

**Methods**

**Culture of primary neonatal rat sympathetic neurons**

Cell culture was performed as previously described (Lockhart et al. 1997). The SCGs were removed from newborn rats and incubated at 37°C for 1 h in 1.5 mg/ml collagenase (Worthington, Lakewood, NJ) and 5 mg/ml dispase (Gibco BRL, Invitrogen, Carlsbad, CA). The ganglia were repeatedly passed through fire-polished glass pipettes to dissociate the tissue. Dissociated SCG cells were then transferred to a plastic culture dish and incubated at 37°C for 2 h to allow attachment of the more adherent Schwann cells and fibroblasts. The dishes were then rinsed and sympathetic neurons were removed and counted. Neurons were plated at 10,000 cells per dish on glass-bottom plates (MatTek, Ashland, MA) coated with collagen (50 mg/ml; BD Biosciences, Bedford, MA) and poly-D-lysine (0.5 mg/ml; BD Biosciences). Cultures were maintained in modified L15-CO2 medium (Hawrot and Patterson 1979; Lockhart et al. 1997), supplemented with 10% fetal bovine serum (Omega Scientific, Tarzana, CA), 6 μM glutamine (Invitrogen), 100 U/ml penicillin/100 μg/ml streptomycin (Invitrogen), 1 μg/ml 6,7, dimethyl-5,6,7,8-tetrahydroypterine (DMPH4, Calbiochem, San Diego, CA), 5 μg/ml glutathione (Sigma, St. Louis, MO), and 100 μg/ml l-ascorbic acid. NGF (5 ng/ml; Upstate, Lake Placid, NY) was added to support neuronal survival and 1 μM cytosine arabinofuranoside (Ara-C, Sigma) was added to inhibit cell division of any flat cells in the culture. Dishes were used for recording on the second to fourth days after culturing.

**Electrophysiology**

Voltage-clamp recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Union City, CA). Current-clamp recordings were performed using an Axoclamp 2B amplifier (Axon Instruments). All recordings were made using patch electrodes (2–6 MΩ) pulled from borosilicate glass (Warner Instruments, Hamden, CT) on a Flaming/Brown horizontal micropipette puller, model P-97 (Sutter Instruments, Novato, CA). The mean series resistance of recordings was 15.5 MΩ, which was routinely compensated by 95%. This typically resulted in calculated voltage errors <2 mV at peak conductance (1.6 ± 0.14 mV). Data traces were acquired using pClamp 8 and a Digidata 1320A digitizer board (Axon Instruments) and were digitized at 10 kHz and low-pass filtered at 2 kHz. All recordings were made at room temperature and the bath was grounded using a 3 M KCl/agar salt bridge.

**Solutions**

Most current-clamp recordings were made using saline containing (in mM): NaCl 126, KCl 3, MgSO4 2, Na2HPO4 1, NaHCO3 25, CaCl2 2; bubbled with 95% O2-5% CO2, and adjusted to 330 mOsmol with dextrose (about 30 mM). Voltage-clamp recordings were performed in external salinie containing of (in mM): NaCl 150, KCl 3, MgCl2 2, Na HEPES 5, CaCl2 2, and dextrose 11; pH 7.4 and adjusted to 330 mOsmol with sucrose. Tetrodotoxin (TTX, 100 nM) and CdCl2 (200 μM) were added to block voltage-dependent sodium and calcium currents, respectively. In experiments using >200 μM CdCl2 MgCl2 was added to the control solution on an equimolar basis to balance the total divalent ion concentration. HEPES-buffered saline was used with cadmium to prevent precipitation, which occurred in the bicarbonate-buffered solution. The internal solution consisted of (in mM): K-glucronate 100, KCl 30, MgSO4 1, CaCl2 1, EGTA 10, Na HEPES 10, and K2ATP 2; pH 7.4 and adjusted to 290 mOsmol with sucrose. The junction potential under our recording conditions was about 12.5 mV and the data were not corrected. NGF was aliquoted and frozen in L15 medium, and added directly to the external saline at a final concentration of 50 ng/ml. NGF is required at low levels (5 ng/ml) in the culture for neurons to survive, so it is not possible to assess the effect of NGF on completely naïve cultures. Therefore our “saline” group represents complete removal of NGF during the period of the recordings and our “NGF” group represents addition of 50 ng/ml NGF to the bath solution.

**Experimental design**

In most of the experiments reported NGF or saline was applied to separate pools of neurons. Making recordings in separate pools of neurons allowed us to compare the effect of saline and NGF at the same time point after the beginning of whole cell recording. Multiple cells were recorded from in each culture dish, which were exposed by bath perfusion to NGF or saline. Voltage- and current-clamp experiments were performed after a mean period of bath perfusion of 64.8 ± 39.1 min for saline (n = 152) and 68.6 ± 30.0 min for NGF (n = 138, mean ± SD). There was no apparent trend between time before recording from a cell and any passive or active property measured in these experiments. For example, the resting membrane potential for cells bathed for 15 to 40 min before recording compared with 80 to 105 min before recording was −53.8 ± 0.7 versus −53.6 ± 0.8 mV for saline, and −57.2 ± 0.9 versus −58.0 ± 0.9 mV for NGF. Action potential amplitudes also did not differ and were 84.1 ± 3.0 versus 81.5 ± 3.5 mV for saline and 79.2 ± 3.4 versus 81.9 ± 2.7 mV for NGF (15–40 min saline, n = 44, NGF, n = 25; for 80–105 min, saline, n = 16, NGF, n = 27).

To confirm the effects of NGF seen in separate pools of neuron we also measured spike output in individual neurons before and after a 15-min application of NGF or saline. After the whole cell configuration was achieved, spike output decreased over the 35–45 min of the experiment with a small but significant decrease in spike number in response to stimulus in the control saline condition (ranging from 15 to 0% across the range of stimulus amplitudes, P = 0.015, two-way ANOVA, n = 13). As described in RESULTS NGF treatment led to a decrease in spike number (ranging from 37 to 5% across the range of stimulus amplitudes, P < 0.001, two-way ANOVA, n = 22) that was significantly greater than the decrease seen in saline alone (two-way ANOVA, P < 0.001). Overall these data demonstrate that time spent in the bath before recording did not affect the firing properties of the neurons. Once a recording was obtained there was a subtle change in firing properties over time that did not preclude our ability to determine the effect of NGF in this paradigm.

**Data analysis and statistics**

Data records were analyzed using Clampfit 8 (pClamp 8, Axon Instruments) and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Current amplitudes measured in voltage-clamp experiments were expressed as current density (pA/pF) to normalize for cell size. Voltage-clamp records were leak subtracted by repeating
protocols at 1/10 the test step amplitude, summing 10 trials, and subtracting the resulting current. Spike threshold was defined as the membrane potential when the first derivative of the voltage trace reached 10 mV/ms. All descriptive measurements of action potentials were made relative to spike threshold [e.g., amplitude, 10–90% amplitude rise and decay times, hyperpolarizing afterpotential (HAP) amplitude, etc.]. Relative changes in HAP amplitude between NGF and saline groups were determined by measuring the HAP elicited.

FIG. 1. Nerve growth factor (NGF) decreases spike output. A: histogram plot showing the percentage of cells firing a particular number of spikes in response to a 440-ms, 170% threshold current pulse; n = 130 for saline and n = 122 for NGF. Response profile was shifted toward lower values in NGF compared with saline. B: similar plot shows that more cells fired fewer spikes in NGF in response to a 290% threshold current pulse. Response profiles in B appear to be bimodal, suggesting the presence of multiple cell populations. C: cumulative probability plot of the same data as in A shows that in NGF a higher percentage of cells fired ≤4 spikes. D: cumulative probability plot of the data presented in B shows the shift to lower spike output and bimodal distribution with 290% threshold current pulses.

FIG. 2. Increased firing frequency but decreased overall spike output in NGF. Voltage traces from a typical saline (top) and typical NGF-treated cell (bottom). Responses are shown for 170, 290, and 500% of threshold current pulses. NGF cell responded to all amplitudes of current pulses by firing fewer spikes, but the frequency for each spike appeared higher than for the saline cell. NGF cell fired quickly and then was silent, whereas the saline cell fired tonically.
between consecutive spikes using sustained current pulses (Baranyi et al. 1993; Castro et al. 2002). The input resistance was measured using a small (40% of threshold current) hyperpolarizing current injection and the membrane time constant was determined by fitting a single-exponential function to the voltage trace. All data points are the average of two to four trials per cell. Data are expressed as means ± SE unless otherwise specified. Data were compared using Student’s $t$-test for normally distributed data sets with equal variance or the Mann–Whitney rank-sum test for data sets that were not normally distributed or had unequal variances, unless otherwise specified in the text (SigmaStat 2.0, Systat Software, Point Richard, CA). Normality was tested using the Kolmogorov–Smirnov test with SigmaStat 2.0.

**RESULTS**

Short-term NGF application decreases spike firing

NGF mediates short-term modulation of sympathetic presynaptic function (Lockhart et al. 1997), suggesting that neurotrophins may regulate repetitive firing and membrane electrical properties in SCG neurons. We therefore measured the neuronal firing properties of sympathetic neurons acutely treated with 50 ng/ml NGF ($n = 122$) or control saline ($n = 130$). We examined firing properties by injecting incremental current steps starting from the threshold current for each cell. We defined the smallest current that elicited at least one action potential in 5/5 trials as the threshold current. Current–voltage relations were determined by injecting a series of test steps ranging from −100 to 500% of threshold current in 30% increments. All cells were allowed to remain at their normal resting potential during this protocol. Comparisons using percentage of threshold current rather than absolute current allowed for variability between individual cells. The threshold current was significantly smaller for saline compared with the NGF group (40.1 ± 1.6 vs. 48.1 ± 2.2 pA, respectively; $P = 0.008$). The resting membrane potential was more depolarized for saline cells compared with NGF (−54.6 ± 0.4 vs. −57.3 ± 0.4 mV, respectively; $P < 0.001$). Neither resting input resistance (543.2 ± 29.0 vs. 522.8 ± 24.2 MΩ, for saline and NGF, respectively) nor membrane time constant differed between groups (40.1 ± 1.8 vs. 37.6 ± 1.7 ms, for saline and NGF, respectively).

We measured spike output by counting the number of spikes elicited with 440-ms depolarizing current steps. The mean spike output was larger in saline than in NGF (3.3 ± 0.1 vs. 2.7 ± 0.1 spikes, respectively, for a 170% threshold pulse, $P < 0.001$; and 5.4 ± 0.1 vs. 4.8 ± 0.2 spikes, respectively, for a 290% threshold pulse, $P = 0.015$). There was no significant difference between groups at 500% threshold pulses, possibly reflecting that pulses of that amplitude push cells toward a physiological limit that obscures the NGF effect. Histogram plots of number of action potentials fired in response to 170 and 290% threshold current pulses are shown in Fig. 1, A and B, demonstrating that in the NGF group neurons fired fewer spikes in response to the stimulus. A cumulative probability

![Graph](http://jn.physiology.org/)

**FIG. 3.** NGF decreased spike output, increased the frequency of the first 3 spikes, and decreased spike time variance. A: plot of times of action potential peaks for all traces recorded from all saline-treated cells is ordered along the y-axis from high spiking (Trial 1) to low spiking (Trial 422) cells ($n = 130$ cells). 422 traces). Times when each consecutive spike occurred relative to pulse onset are indicated by different symbols (legend, top). Data represent at least 2 and no more than 4 trials for each cell and the stimulus was 170% of threshold current. B: similar plot for NGF-treated cells ($n = 122$ cells, 474 traces) shows that there were fewer cells that fired multiple spikes in NGF compared with saline (A). C and D: bar plot of mean spike times (C) and variance of spike times (D) for the data shown in A and B shows that spike times were earlier in NGF (open bars) compared with saline (filled bars) for the first 3 spikes (1st spike: saline $n = 130$ and NGF $n = 122$; 2nd spike: saline $n = 120$ and NGF $n = 95$; 3rd spike: saline $n = 97$ and NGF $n = 99$). D: plot of the variance, or absolute deviation from the mean, for the 1st and 2nd spikes and the 1st and 2nd interspike intervals (ISIs) for cells firing 2 or 3 action potentials. Spike time variance was significantly decreased for the 1st 2 spikes and the variance of the 1st, but not the 2nd ISI was also decreased in NGF (open bars) compared with control (closed bars): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.**
NGF decreases spike latency and interspike interval

The NGF-dependent increase in frequency was also observed with 290 and 500%, as well as 170%, threshold current pulses. This is shown by a decrease in the spike latency (i.e., defined as time between consecutive action potential peaks. ISI was defined as the time from pulse onset to the peak of the first spike. ISI was plotted in descending order along the y-axis from trials with one spike (Fig. 3, A and B). Data from at least two and no more than four trials are shown for each cell (saline, 130 cells, 422 trials; and NGF, 122 cells, 474 trials). Trials are plotted in descending order along the y-axis from trials with seven spikes (Trial 1) to trials with one spike (Trial 422 or 474; Fig. 3, A and B). It can be seen from these plots that fewer cells fired multiple action potentials in NGF compared with control (Fig. 3, A and B). Additionally, action potentials occurred significantly earlier with respect to the onset of the pulse in NGF compared with saline for the first three spikes as can be seen in Fig. 3, A and B and plots of the mean spike times for the first three spikes (Fig. 3C).

The variance of the spike times (the absolute deviation from the mean for each data point) was also decreased in NGF. This was statistically significant for the first two spikes for all cells firing two or more action potentials (7.2 ± 0.3 vs. 5.5 ± 0.7 ms, P < 0.001; and 21.0 ± 0.9 vs. 17.0 ± 0.9 ms, P = 0.004, for saline vs. NGF and for the first and second spike, respectively; Fig. 3D). The first interspike interval (ISI) also showed significantly less variance in NGF compared with saline (13.5 ± 0.8 vs. 15.6 ± 1.6 ms, respectively; P = 0.025; Fig. 3D). Thus acute NGF treatment caused SCG neurons to fire with a higher initial frequency and a higher degree of coincidence relative to stimulus onset. These changes could potentially lead to an amplification of signal in vivo by temporally concentrating the activity of a population of neurons.

NGF alters the repetitive firing pattern of SCG neurons

Although NGF decreased spike output it caused an increase in the firing frequency. Voltage traces for a typical sympathetic neuron recorded from in saline or in NGF are shown in Fig. 2, top and bottom, respectively. In this example the NGF cell fired two spikes in response to a 170% threshold current pulse and then did not fire again for the remainder of the pulse, whereas the saline cell fired three spikes that were spread relatively evenly across the duration of the stimulus. A similar pattern was seen with 290 and 500% pulses. NGF appears to promote a phasic response characterized by a rapid initial firing followed by a silent period, whereas cells in saline tended to fire tonically.

The shift to a more phasic pattern of firing in NGF is seen more clearly in plots of the time of action potential peaks relative to the onset of a 440-ms pulse of 170% threshold current amplitude (Fig. 3, A and B). Data from at least two and no more than four trials are shown for each cell (saline, 130 cells, 422 trials; and NGF, 122 cells, 474 trials). Trials are plotted in descending order along the y-axis from trials with seven spikes (Trial 1) to trials with one spike (Trial 422 or 474; Fig. 3, A and B). It can be seen from these plots that fewer cells fired multiple action potentials in NGF compared with control (Fig. 3, A and B). Additionally, action potentials occurred significantly earlier with respect to the onset of the pulse in NGF compared with saline for the first three spikes as can be seen in Fig. 3, A and B and plots of the mean spike times for the first three spikes (Fig. 3C).

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time from stimulus onset to the first spike) and ISI (Fig. 4). Spike latency was significantly decreased in NGF with pulses of 170% of threshold current (31.9 ± 1.0 vs. 26.9 ± 0.7 ms, for saline and NGF, respectively; \( P < 0.001 \)), 290% of threshold current (17.5 ± 0.5 vs. 15.1 ± 0.4 ms, for saline and NGF, respectively; \( P < 0.001 \)), and 500% of threshold current (10.2 ± 0.3 vs. 9.1 ± 0.2 ms, for saline and NGF, respectively; \( P < 0.001 \); \( n = 130 \) for saline and \( n = 122 \) for NGF; Fig. 4B). The first ISI was also decreased in NGF with 170% threshold current pulses (93.0 ± 2.0 vs. 84.6 ± 1.6 ms, for saline and NGF, respectively; \( P = 0.002 \)), with small but significant decreases also seen with 290% threshold current pulses (56.4 ± 1.1 vs. 53.2 ± 1.2 ms, for saline and NGF, respectively; \( P = 0.012 \)), and 500% of threshold current (37.4 ± 0.8 vs. 35.6 ± 0.7 ms, for saline and NGF, respectively; \( P = 0.038 \); \( n = 120 \) for saline and \( n = 95 \) for NGF; Fig. 4C). The second ISI was shorter in NGF with 170%, but not 290 or 500%, threshold current pulses (112.9 ± 2.8 vs. 103.6 ± 2.7 ms, for saline and NGF, respectively; \( P = 0.040 \); \( n = 97 \) for saline and \( n = 59 \) for NGF; data not shown).

**NGF causes a hyperpolarization of spike threshold and decreases the duration and amplitude of the hyperpolarizing afterpotential**

The properties of the first and second action potentials were not markedly different, although the threshold potential of the first spike was more hyperpolarized for NGF compared with saline control (Table 1). This is consistent with the observed decrease in spike latency. We also observed a small but significant decrease in the amplitude and duration of the hyperpolarizing afterpotential (HAP) relative to control (Fig. 5). Relative changes in HAP properties were determined using 440-ms pulses of 170% threshold current. There was a 10% decrease in both the half-amplitude duration (55.6 ± 1.6 vs. 49.9 ± 1.2 ms, saline vs. NGF, \( P < 0.001 \)), and 90–30% decay times (58.8 ± 1.6 vs. 52.6 ± 1.3 ms, saline vs. NGF, \( P = 0.004 \)) in NGF compared with saline. The HAP amplitude decreased by 5.5% in NGF compared with control (−30.9 ± 0.4 vs. −29.2 ± 0.4 mV, saline vs. NGF, \( P = 0.002 \); \( n = 130 \) for saline and \( n = 120 \) for NGF).

**NGF decreases the amplitude of calcium-independent potassium currents in SCG neurons**

Voltage-gated potassium currents are important in regulating repetitive firing properties (Belluzzi et al. 1985; Fernandez et al. 2005; Mo et al. 2002; Ozaita et al. 2004) and are modulated by NGF in rat sensory neurons (Zhang et al. 2002). We therefore performed voltage-clamp experiments to determine whether modulation of potassium currents contribute to NGF-mediated alterations of repetitive firing in SCG neurons.

We performed voltage-clamp experiments in the presence of 100 nM TTX and 200 μM CdCl₂, which block sodium and calcium currents, respectively, to examine calcium-independent potassium currents. We separated currents into three groups based on inactivation properties: rapidly inactivating, slowly inactivating, and noninactivating currents. These presumably correspond to the previously described rapidly inactivating and slowly inactivating A-currents and delayed rectifier current, respectively (McFarlane and Cooper 1992). Voltage-clamp steps (−80 to +40 mV) made from −110 mV activated a family of mixed outward current components (Fig. 6A). A short depolarizing prepulse preferentially removed a rapidly inactivating component, leaving more slowly and noninactivating components (Fig. 6B). The rapidly inactivating component was isolated by subtracting the traces generated with the prepulse from those generated without the prepulse (Fig. 6, A minus B, right). Holding cells at −10 mV inactivated both the rapidly and slowly inactivating currents and test steps resulted in traces consisting of the noninactivating component and leak current (Fig. 6C). The leak current was subsequently subtracted out (see METHODS), isolating the noninactivating current (Fig. 6C, p/10 leak subtraction, right). The slowly inactivating component was isolated by subtracting currents generated from a holding potential of −10 mV from currents

![NGF ALTERS POTASSIUM CURRENTS AND REPETITIVE FIRING](image-url)

**FIG. 5. Measurement of hyperpolarizing afterpotential (HAP) parameters.** A typical voltage trace is shown for a saline (black) and an NGF-treated cell (gray). Amplitude of the HAP after each spike was defined as the maximum negative voltage deflection relative to threshold and between consecutive action potentials. Half-amplitude duration and the 90–30% amplitude decay times were defined relative to the peak amplitude (arrows).

![Table 1](table-url)

**TABLE 1. Action potential properties**

<table>
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<tr>
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<th>AP Peak, mV</th>
<th>Rise, ms</th>
<th>Decay, ms</th>
<th>Half-Duration, ms</th>
<th>Threshold, mV</th>
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<tr>
<td><strong>First action potential</strong></td>
<td></td>
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<tr>
<td>Saline (( n = 130 ))</td>
<td>81.5 ± 1.5</td>
<td>0.71 ± 0.01</td>
<td>2.98 ± 0.07</td>
<td>2.58 ± 0.07</td>
<td>−27.9 ± 0.5**</td>
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<tr>
<td>NGF (( n = 122 ))</td>
<td>82.2 ± 1.3</td>
<td>0.69 ± 0.01</td>
<td>3.15 ± 0.10</td>
<td>2.62 ± 0.08</td>
<td>−29.4 ± 0.4**</td>
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<tr>
<td><strong>Second action potential</strong></td>
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<tr>
<td>Saline (( n = 120 ))</td>
<td>74.4 ± 1.7</td>
<td>0.84 ± 0.02</td>
<td>3.10 ± 0.09</td>
<td>2.85 ± 0.08</td>
<td>−24.7 ± 0.4</td>
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<tr>
<td>NGF (( n = 95 ))</td>
<td>71.7 ± 1.4</td>
<td>0.83 ± 0.02</td>
<td>3.25 ± 0.11</td>
<td>2.98 ± 0.10</td>
<td>−25.5 ± 0.5</td>
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Values are means ± SE, measured relative to spike threshold. AP peak, action potential peak. Rise and decay are 10–90% peak amplitude rise and decay times, respectively. Half-duration is the duration at half-peak amplitude. Threshold is the membrane potential when the slope of the voltage trace reached 10 mV/ms (\( P = 0.003 \)). All values were measured with 170% threshold current pulses given from resting potential. **\( P < 0.01 \).
generated by stepping from −110 mV with the depolarizing prepulse (Fig. 6, B minus C, right).

NGF decreased the amplitude of all three kinetically separated current components. As previously described (see METHODS), separate pools of neurons were recorded from in the presence of NGF (50 ng/ml, n = 16) or in control saline (n = 44). Representative traces generated with +20-mV steps in saline or NGF are shown in Fig. 7A. We compared the total current density, expressed as the area of leak-subtracted current traces generated with steps from −110 mV and no prepulse (Fig. 7B). The rapidly inactivating current density was measured at peak for each trace (Fig. 7C). The slowly inactivating current density was measured as the mean value between 80 and 130 ms after test step onset (a time frame during which the rapid component was inactivated and therefore unlikely to cause contamination of the current traces, yet the slow component was near peak; Fig. 7D). The noninactivating current density was measured over the last 50 ms of the test step (Fig. 7E). The total current and all three kinetically separable components were significantly reduced in NGF.

The activation voltage dependency of the three kinetically separated current components differed from each other, but did not change in response to NGF (Fig. 8). Activation voltage dependency was determined by fitting Boltzmann functions to plots of chord conductance \([I/(V_{\text{step}} - V_{\text{reversal}})]\) versus test step amplitude. The Boltzmann fit provides parameters describing the voltage dependency of channel activation. The half-activation is the midpoint of the activation curve and the slope factor \(\tau\) describes the slope of the linear portion of the \(G-V\) curve. The rapidly inactivating current had the most hyperpolarized voltage dependency of activation (Boltzmann-fit parameters: \(V_{1/2}: -11.4 \pm 1.1\) vs. \(-13.8 \pm 1.2\) mV and \(\tau: 11.3 \pm 0.4\) vs. 12.2 ± 0.7 ms, for saline and NGF, respectively). The slowly inactivating current had the most depolarized voltage dependency of activation (Boltzmann-fit parameters: \(V_{1/2}: 8.1 \pm 1.2\) vs. 10.7 ± 1.6 mV and \(\tau: 9.4 \pm 0.3\) vs. 10.2 ± 0.8 ms, for saline and NGF, respectively). The noninactivating component had Boltzmann-fit parameters of \(V_{1/2}\) of \(-4.4 \pm 2.0\) versus \(-3.0 \pm 2.2\) mV and \(\tau\) of 14.9 ± 0.6 versus 13.8 ± 0.6 ms, for saline and NGF, respectively. Half-activation voltages were significantly different for the three conductances \((P < 0.001; \text{ANOVA, followed by Student–Newman–Keuls test for pairwise comparison})\). Slope factors \(\tau\) were also all significantly different from each other \((P < 0.05; \text{ANOVA on ranks, followed by Dunn’s test for pairwise comparison})\). Comparisons between saline and NGF groups yielded no statistically significant differences.

**NGF decreases the amplitude of calcium-dependent potassium currents in SCG neurons**

Calcium-dependent potassium currents \((I_{\text{KCa}})\) play a role in shaping the action potential, the hyperpolarizing afterpotential, and determining repetitive firing properties in many types of neurons including SCG neurons (Davies et al. 2006; Faber and Sah 2002; Sacchi et al. 1995). Additionally, they appear to be developmentally regulated by NGF in chick sympathetic neurons (Raucher and Dryer 1995). We therefore performed voltage-clamp experiments to determine whether NGF
FIG. 7. NGF decreased voltage-gated potassium current density. A: representative traces of the 3 kinetically separable calcium-independent potassium currents show decreased amplitude in NGF. Traces show current density (pA/pF) for voltage-clamp records made from cells in saline (black) or 50 ng/ml NGF (gray). Top: rapidly inactivating component. Middle: slowly inactivating component. Bottom: noninactivating component. Representative traces are shown for test steps to +20 mV. B: total current was measured as the area under the leak subtracted current trace (inset, shaded) elicited with steps from −110 mV to test steps ranging from −80 to +40 mV (−60 to +40 mV shown). Plot of the total current density against test step potential shows that the current was decreased in NGF (open triangles) compared with saline (filled circles; P = 0.019, two-way ANOVA). C: rapidly inactivating component was measured at peak for each trace (inset) and was significantly decreased by NGF (P = 0.011, two-way ANOVA). D: slowly inactivating component was expressed as the mean current between 80 to 130 ms after the test step (inset) and was significantly decreased in NGF compared with saline (P = 0.023, two-way ANOVA). E: noninactivating component amplitude was expressed as the mean current over the last 50 ms of a 1.8-s test step (inset) and was also decreased across the voltage range tested (P = 0.006, two-way ANOVA; n = 44 for saline and n = 16 for NGF).

acutely affected SCG neuronal firing pattern through modulation of $I_{\text{KCa}}$.

We matched the temporal design of our current-clamp protocols by using a protocol to measure $I_{\text{KCa}}$ that was fast and did not require digital subtraction or wash-in of pharmacological agents. We used the tail current, the small current occurring after a depolarizing test step, to measure $I_{\text{KCa}}$ (Fig. 9A) (Cassell and McLachlan 1987; Davies et al. 1996; Goldberg and Wilson 2005). The $I_{\text{KCa}}$ deactivates more slowly than other potassium currents and therefore is the major contributor to the latter part of the tail current in SCG neurons (Sacchi et al. 1995). We measured the tail current over a 5-ms window after a 500-ms step to −10 mV from a holding potential of −60 mV. Neither the rapidly or slowly inactivating calcium-independent currents would be expected to significantly contribute to the tail current generated with this protocol because the former would completely inactivate during the test step and the threshold for activation for the latter is near −10 mV (Figs. 9B and 8). The noninactivating component should deactivate more rapidly than $I_{\text{KCa}}$, and would be expected to contribute less to the tail during the time frame used here (Sacchi et al. 1995).

Indeed our evidence suggests that the tail current recorded under these conditions is largely carried by $I_{\text{KCa}}$. We found that the tail current had a significantly different current–voltage relationship than the slowly and noninactivating but not the rapidly inactivating calcium-independent current components described above (Boltzmann-fit parameters for $I_{\text{KCa}}$: $V_{1/2}$: −14.7 ± 1.7 mV; $\tau$: 13.0 ± 1.4 ms; $n = 13$; Fig. 9B). The half-activation voltage was significantly different with $P < 0.001$ for all values except the rapid component versus tail current, which was not significantly different (ANOVA, followed by Student–Newman–Keuls test for pairwise comparison). The slope factor ($\tau$) was significantly different with $P < 0.05$ for all values except the rapid versus tail and noninactivating versus tail (ANOVA on ranks, followed by Dunn’s test for pairwise comparison). These values for activation voltage dependency agree with those already reported for calcium-dependent potassium currents in SCG neurons (Belluzzi and Sacchi 1990).

The pharmacological sensitivity of the tail current also suggests that it is primarily carried by $I_{\text{KCa}}$. A 7-min application of 1–2 mM CdCl$_2$, a calcium channel blocker, caused a significantly larger decrease than either saline or the voltage-gated potassium channel blocker 4-aminopyridine (4-AP, 4–6 mM; Fig. 9C). The effects of saline and 4-AP were not significantly different from each other (current density normalized to control: 0.44 ± 0.05 for CdCl$_2$, compared with 0.85 ± 0.04 and 0.72 ± 0.07 for saline and 4-AP, respectively; saline vs. CdCl$_2$, $P < 0.001$ and 4-AP vs. CdCl$_2$, $P = 0.002$; ANOVA, followed by Student–Newman–Keuls test for pair-
wise comparison; Fig. 9C). We found that 2 mM 4-AP reduced total calcium-independent potassium current in SCG neurons by >50% using the protocols described in the last section (n = 3; data not shown).

Although the \( I_{KCa} \) is the major component of the tail current, we would expect that current measured at the end of the step would contain relatively large contributions from calcium-independent potassium currents as well as from \( I_{KCa} \). Consistent with this idea, we found that current measured at the end of the pulse was decreased by a similar extent with application of either CdCl\(_2\) or 4-AP (current density normalized to control 0.93 ± 0.04 for saline, 0.58 ± 0.06 for CdCl\(_2\), and 0.69 ± 0.05 for 4-AP; saline vs. CdCl\(_2\), \( P < 0.001 \), saline vs. 4-AP, \( P = 0.006 \) and CdCl\(_2\) vs. 4-AP was not significantly different; ANOVA, followed by Student–Newman–Keuls test for pairwise comparison; Fig. 9D). This suggests that the current at the end of the step constitutes both \( I_{KCa} \) and calcium-independent components and that the tail current is mostly carried by \( I_{KCa} \).

The amplitude of the tail current was significantly smaller in NGF compared with saline, but the amplitude of the current measured at the end of the test step did not differ (Fig. 10, A and B; 11.9 ± 0.9 vs. 7.8 ± 0.8 pA/pF for saline and NGF tail current, respectively; \( P = 0.003 \), and 36.6 ± 2.6 vs. 32.3 ± 2.5 pA/pF for saline and NGF measured at the end of the step, respectively; saline, \( n = 25 \) and NGF, \( n = 17 \)). It should be pointed out that we would not expect to see significant differences in the NGF-induced calcium-independent currents using this protocol because those differences were strongest with steps more depolarized than −10 mV (see Fig. 7).

Our findings demonstrate that NGF decreases both the total number of spikes and \( I_{KCa} \). We therefore performed current-clamp experiments to determine how blocking \( I_{KCa} \) using 200 \( \mu \)M CdCl\(_2\) would affect repetitive firing properties in our system. We found that, similar to treatment with NGF, CdCl\(_2\) caused a reversible decrease in spike output in response to a 170% of threshold current stimulus pulse (3.8 ± 0.2 vs. 2.9 ± 0.3 spikes for saline and CdCl\(_2\), respectively, \( n = 11 \); \( P = 0.019 \), paired \( t \)-test; Fig. 11, A and B). Also similar to NGF treatment, amplitudes of the first and second HAPs were reversibly decreased by CdCl\(_2\) (−30.7 ± 1.1 vs. −26.6 ± 1.3 mV for the first HAP; \( P = 0.002 \), and −30.8 ± 1.1 vs. −25.5 ± 1.1 mV for the second HAP; \( P < 0.001 \), for saline and CdCl\(_2\), respectively; \( n = 11 \); paired \( t \)-test; Fig. 11C).

Application of CdCl\(_2\) did not reproduce all aspects of the NGF effect, suggesting the involvement of other currents besides \( I_{KCa} \). The duration of the first ISI or HAP was not significantly different after CdCl\(_2\) application (101.6 ± 3.8 vs. 111.5 ± 12.3 ms for the first ISI and 63.5 ± 2.8 vs. 71.9 ± 7.6 ms for the first HAP half-amplitude duration, for saline and CdCl\(_2\), respectively). The spike latency was also not significantly altered by CdCl\(_2\) (33.8 ± 1.5 vs. 39.6 ± 3.8 ms for spike latency, for saline and CdCl\(_2\), respectively).

**FIG. 8.** Activation voltage dependency for the 3 kinetically separable calcium-independent potassium currents was unaffected by NGF. Plots of normalized chord conductance [\( h(V_{mep} - V_{KCa,rev}) \)] show that the voltage dependency of activation was unaltered by 50 ng/ml NGF for the rapidly inactivating (A), the slowly inactivating (B), and the noninactivating current components (C). Half-amplitude voltages were \( V_{1/2} \): −11.4 ± 1.1 vs. −13.8 ± 1.2 mV, 8.1 ± 1.2 vs. 10.7 ± 1.6 mV, and −4.4 ± 2.0 vs. −3.0 ± 2.2 mV for rapid, slow, and noninactivating components, and for saline vs. NGF, respectively. Slope factors (\( r \)) were: 11.3 ± 0.4 vs. 12.2 ± 0.7 ms, 9.4 ± 0.3 vs. 10.2 ± 0.8 ms, and 14.9 ± 0.6 vs. 13.8 ± 0.6 ms, for rapid, slow, and noninactivating components, and for saline vs. NGF, respectively.

**Discussion**

NGF release from heart and other tissues in vivo is regulated by physiologically relevant stimuli and pathological states (Bjorling et al. 2002; Hiltunen et al. 2001; Kaye et al. 2000; Peeraully et al. 2004; Qin et al. 2002). Sympathetic synaptic properties are rapidly influenced by NGF (Lockhart et al. 1997) and here we have demonstrated that NGF also regulates firing
pattern and potassium currents within a 2-h period. We show that, in vitro, NGF rapidly decreases spike output but increases frequency. NGF reduces the amplitude of both $I_{\text{KCa}}$ and calcium-independent potassium currents, and inhibition of $I_{\text{KCa}}$ reproduces some effects of NGF, including the decrease in total spike number. These data suggest that rapid regulation of potassium channels by NGF contributes to the regulation of firing properties by target-derived factors. Thus variable NGF signaling from target tissue may be a mechanism regulating sympathetic membrane electrical and synaptic properties.

**Sympathetic neurons** can be classified into either phasic or tonic groups based on their repetitive firing pattern. Phasic neurons fire a burst of action potentials in response to a continued stimulus, whereas tonic neurons fire continuously.

**FIG. 10.** Calcium-dependent potassium current was reduced by NGF. A: representative voltage-clamp traces recorded from a cell exposed to saline (black trace) and NGF (gray trace) in response to a 500-ms step from −60 to −10 mV. B: bar plot of tail current amplitude shows that calcium-dependent potassium current is decreased in NGF (open bar, $n = 17$) compared with saline (filled bar, $n = 25$, $P = 0.003$). C: bar plot showing that the current measured at the end of the step was not significantly different in NGF (open bar, $n = 17$) compared with saline (filled bar, $n = 25$).

**FIG. 9.** Method for measurement of calcium-dependent potassium current amplitude. A: voltage-clamp record of current density (pA/pF) made with a 500-ms step to −10 mV from a −60-mV holding potential. Tail current (small current after test step offset) was used to measure calcium-dependent potassium current amplitude and was averaged over a 5-ms window 10 ms after the end of the step. We also measured the current just before the end of the step, averaged over a 40-ms window. B: plots of normalized current density (for tail current; $n = 13$) or normalized chord conductance (for calcium-independent components; $n = 44$ for each) vs. test step amplitude show that the tail current had a more hyperpolarized voltage dependency of activation compared with slowly and noninactivating calcium-independent current components, but not the rapid component. C: pharmacology of the tail current was consistent with calcium-dependent current. Bar plot of normalized current density shows that the tail current was more strongly blocked by the calcium channel blocker 1–2 mM CdCl$_2$ (open bar) than the voltage-gated potassium channel blocker 4–6 mM 4-aminopyridine (4-AP; gray bar). D: bar plot of current amplitude measured at the end of the step shows that amplitude was similarly decreased by both CdCl$_2$ (open bar) and 4-AP (gray bar) compared with saline (filled bar) (saline, $n = 12$; CdCl$_2$, $n = 12$; and 4-AP $n = 6$; ANOVA, followed by Student–Newman–Keuls test for pairwise comparison; **$P < 0.01$ and ***$P < 0.001$).
Under our culture conditions, SCG neurons show tonic firing properties, although these neurons tend to be exclusively phasic when examined in the intact ganglia (Jobling and Gibbins 1999; Wang and McKinnon 1995). Interestingly, NGF treatment was sufficient to shift cultured SCG neurons from a tonic to a phasic firing pattern. Neurons exposed to NGF tended to fire fewer action potentials more rapidly, whereas neurons in saline tended to fire tonically. Thus NGF signaling from in vivo targets may regulate firing pattern and promote the phasic phenotype commonly observed in the SCG. We also found that NGF significantly decreased the spike time variance of the first action potentials and the associated interspike interval in neurons exposed to NGF. This suggests that NGF may act in vivo to cause a population of sympathetic neurons to fire with a higher degree of coincidence, thus temporally amplifying sympathetic output to the heart or other targets.

The pattern of spike discharge is an important determinant of neurotransmitter and neuropeptide release from neurons. For example, adrenal chromaffin cells release catecholamines, but under conditions of increased sympathetic activity they also release neuropeptides in an manner that is dependent on the pattern of sympathetic input (Fulop et al. 2005). Brain-derived neurotrophic factor (BDNF) is released from hippocampal neurons in a manner that is dependent on the frequency and pattern of activity (Balkowiec and Katz 2002). Thus NGF-mediated changes in the sympathetic neuronal firing pattern (i.e., phasic vs. tonic) provide a potential mechanism to increase efficacy of norepinephrine release at noradrenergic synapses in the heart.

Many studies have linked the types and properties of potassium currents to a neuron’s expression of its firing patterns (Rudy 1988). We found that NGF decreased the amplitude of both calcium-dependent and calcium-independent potassium currents. Like NGF, the calcium channel blocker CdCl₂ caused a decrease in 

\[ I_{K_{Ca}} \]

and spike output, suggesting that 

\[ I_{K_{Ca}} \]

contributes to changes in spike number in response to NGF. Both NGF and CdCl₂ decreased the amplitude of the HAP, suggesting that 

\[ I_{K_{Ca}} \]

also contributes to the HAP. However, unlike NGF, CdCl₂ did not cause significant changes in spike latency or interspike interval, suggesting that, although 

\[ I_{K_{Ca}} \]

contributes to the NGF-dependent regulation of firing properties, NGF is also likely to regulate additional currents. Consistent with this conclusion, we also observed the NGF-mediated decrease in calcium-independent potassium currents, which are involved in determining spike latency and interspike interval (Belluzzi and Sacchi 1988, 1991; Belluzzi et al. 1985; Connor and Stevens 1971; Liss et al. 2001; Rudy 1988). NGF-induced decreases in these currents are thus consistent with the observed decreases in spike latency and ISI. A reduction in these calcium-independent potassium currents could also explain the observed hyperpolarization of the spike threshold potential because activation of these currents opposes depolarization and could shift threshold.

Some of the effects of NGF on the SCG neurons cannot be explained solely by the observed decreases in potassium currents. Reduction in potassium currents does not account for the increase in threshold current or hyperpolarization of resting membrane potential observed in NGF. Most likely other currents not investigated here are also modulated by NGF in SCG neurons. For example, NGF induces synthesis of new calcium channels in developing hippocampal neurons (Baldelli et al. 2000) and upregulates sodium current in rat sensory neurons (Zhang et al. 2002). BDNF downregulates K⁺–Cl⁻ cotransporter activity in hippocampal neurons, reducing Cl⁻ ion extrusion (Rivera et al. 2002; Wardle and Poo 2003). NGF may modulate 

\[ Cl^- \]

transporters in SCG neurons, which would influence both expressed chloride currents (Clark et al. 1998; De Castro et al. 1997; Sacchi et al. 1999) and membrane electrical properties. Therefore the regulation of firing patterns by NGF is likely to involve other conductances and future studies will be required to elucidate the exact mechanisms.

It is interesting that the NGF-mediated decreases in potassium current do not lead to greater effects on action potential waveform. One possible explanation is that sodium current inactivation may be the predominating determinant of spike repolarization under our conditions. Another possibility may be that potassium channel subunits in the axonal compartment, presumably contributing to spike waveform, are differentially modulated by NGF. Potassium channel subtypes are located heterogeneously in clusters in specific neuronal regions (Hoffman et al. 1997; Kollo et al. 2006). Because of technical considerations our voltage-clamp records probably reflect currents of somatic origin only, so we do not know how currents in other compartments may be modulated by NGF. If potassium currents in the axon are differentially regulated by NGF then the spike waveform might be unaffected even though somatic currents are significantly decreased.

The responses of SCG neurons to neurotrophins change over developmental time. During development, sympathetic neurons acquire dependency on NGF after a period of neurotrophin-3 responsiveness (Birren et al. 1993; DiCicco-Bloom et al. 1993). These changes are likely to influence the developmental expression patterns of the currents responsible for the
acquisition of mature functional properties. In addition to target regulation of calcium-dependent potassium current in embryonic chick sympathetic and parasympathetic neurons (Cameron et al. 2001; Lhuillier and Dryer 2002; Raucher and Dryer 1995), neurotrophins regulate potassium channels during postnatal development. For example, expression of rapidly and slowly inactivating potassium currents in cultured postnatal SCG neurons is dependent on ciliary neurotrophic factor, which is secreted from nonneuronal ganglionic cells (McFarlane and Cooper 1992, 1993). The rapidly inactivating current loses dependency on extrinsic factors after postnatal day 14, but the slowly inactivating component remains dependent (McFarlane and Cooper 1993). Here we show that rapid NGF-mediated regulation of sympathetic neuronal firing patterns and potassium currents can occur during postnatal periods. In future studies it will be important to determine whether NGF modulation of sympathetic membrane properties is differentially regulated over later postnatal development.

Our data suggest that NGF signaling from target tissue regulates repetitive firing properties of sympathetic neurons on a relatively rapid timescale. Sympathetic neurons display distinct electrical, morphological, and synaptic properties that are correlated to the type of target tissue that they innervate (Anderson et al. 2001; Arbab et al. 1986; Flett and Bell 1991; Kukula and Szelcny 1998; Li and Horn 2006; Szelcny et al. 2006). Differential expression of neurotrophic factors in different target tissues is an important regulator of sympathetic neuronal properties as demonstrated by the acquisition of cholinergic properties in vasoconstrictor neurons forced to innervate sweat glands, an inappropriate target (Koltzunberg et al. 1996). Sweat pads produce multiple cholinergic differentiation factors, suggesting that target-derived signaling is important in regulating neurotransmitter phenotype of sympathetic neurons in vivo (Rao et al. 1992). This provides an example of target-specific signals influencing the phenotype of mature neurons. We have shown that NGF regulates voltage-gated and calcium-dependent potassium channels and the repetitive firing pattern of SCG neurons, suggesting a mechanism by which the heart may regulate the functional properties of innervating neurons. Release of NGF from heart and other tissues in vivo is physiologically regulated and state dependent (Björing et al. 2002; Hiltunen et al. 2001; Kaye et al. 2000; Peeraully et al. 2004; Qin et al. 2002) and thus may be an important regulatory signal controlling sympathetic neuronal properties.

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