ORIGINAL RESEARCH

# The Effects of Nerve Growth Factor on Nicotinic Synaptic Transmission in Mouse Airway Parasympathetic Neurons

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## Abstract

In autonomic ganglia, acetylcholine (ACh) is released from preganglionic nerve terminals and binds to nicotinic ACh receptors (nAChRs) on postganglionic neurons, resulting in a brief, short-lived synaptic potential (fast excitatory postsynaptic potential [fEPSP]). Although nerve growth factor (NGF) is known to affect sensory and sympathetic nerves, especially during development, little is known regarding its effect on parasympathetic nerves, especially on adult neurons. Elevated levels of NGF and NGF-mediated neural plasticity may have a role in airway diseases, such as asthma and chronic obstructive pulmonary disease. In this study, we characterize the composition and response of nAChRs in parasympathetic neurons located in lower airways of mice, and note the effects of NGF on fEPSPs and on nicotinic currents. Based on immunohistochemical staining, nAChRs are made up of α-3 and β-4 subunits; in addition, tropomyosin-related kinase A, the receptor for NGF, is also expressed by the neurons. Vagus nerve evoked fEPSPs and inward currents evoked by a nicotinic receptor agonist (1,1-dimethyl-4-phenylpiperazinium) were increased by NGF. NGF also affected the action potential after hyperpolarization. These studies were done in mice, which are routinely used to study airway diseases, such as asthma, where the allergen-induced contraction of airway smooth muscle has a well-defined parasympathetic cholinergic component.

**Keywords:** nerves; parasympathetic; cholinergic; bronchoconstriction; asthma

### Clinical Relevance

In chronic allergy, asthma, and chronic obstructive pulmonary disease, the airways are remodeled with increased growth or number of infiltrating cells, fibroblasts, smooth muscle, and glands. All of these are potential sources of neurotrophins and related neurotrophic factors that may affect nearby parasympathetic nerves. The long-term objective of our research is to determine the mechanism of action of neuronal growth factors that affect the airway nervous system, particularly autonomic parasympathetic neurons. Results of our research should provide critical insights into how neurotrophic growth factors cause dysfunction of parasympathetic neurons, especially during airway inflammation and disease.

Neurons in airway parasympathetic ganglia function to control and distribute the signals emanating from the central nervous system (CNS) and are thus pivotal in regulating airway smooth muscle tone and airway caliber (1) In the parasympathetic pathway, preganglionic nerves from the CNS brainstem terminate at synapses within parasympathetic ganglia near the airway wall; postganglionic axons exit these ganglia and innervate the entire airway tree, including bronchioles (2). Nicotinic acetylcholine (ACh) receptors (nAChRs) are known to mediate fast synaptic transmission in all autonomic ganglia; ACh, released from preganglionic nerve terminals, binds to nAChRs on postganglionic neurons, resulting in a brief, short-lived synaptic potential (fast excitatory postsynaptic potential [fEPSP]). Although much is known about nAChRs in sympathetic ganglia, little is known about this receptor in mammalian parasympathetic ganglia (3). Modification of nAChRs by alterations in number, binding, or ion gating could greatly affect how individual neurons relay information from the CNS (i.e., how neurons integrate presynaptic information).

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The family of neuronal growth factors referred to as neurotrophins (NTs) consists of polypeptides that support growth, differentiation, and survival of neurons in developing and adult nervous systems (4). The first-discovered and prototypical NT was nerve growth factor (NGF) (5); the other known NTs are brain-derived neurotrophic factor (BDNF), NT-3, and NT-4/5 (reviewed in Ref. 4). NTs act on a family of receptor tyrosine kinases first identified as tropomyosin-related kinase (Trk) receptors. NGF preferentially activates TrkA receptors, BDNF and NT-4/5 are selective for TrkB receptors, and NT-3 is selective for TrkC receptors (4). The activation of these NT receptors (NTRs) involves ligand-induced homodimerization, activation of intrinsic Trk receptor kinase activity by autophosphorylation, and phosphorylation of juxtaposed receptors, leading to the activation or modulation of multiple signal transduction pathways. NTs may also bind to an additional NTR (p75NTR) that binds all the NTs, albeit with no specificity, with lower affinity, and no resultant kinase activity as compared with NT Trk receptors (4). Adult nerves have either a single high-affinity receptor or different combinations of high-affinity receptors, usually with varying levels of the low-affinity receptor (6, 7).

The NT, NGF, is increased in asthmatic airways (8) and affects the airway nerve system (9–12). In mice, levels of NGF have been reported to be increased in infiltrating cells, epithelial cells, and airway smooth muscle after allergic inflammation (13), and is associated with enhanced allergic response (14, 15) and airway remodeling (16). It has been known for some time that NGF has a rapid neurotransmitter-like action on sensory neurons (e.g., Ref. 17), but determining the acute effects of NTs on autonomic neurons is a relatively understudied area of investigation (11). In this report, we provide evidence that the NGF-selective receptor, TrkA, is expressed by principal parasympathetic ganglionic neurons in mouse lower airways, and that NGF alters electrophysiological and synaptic properties of ganglionic neurons, alterations that may decrease integration (filtering) by these neurons.

## Materials and Methods

### Animals

The methods for animal use were approved by the Johns Hopkins Animal Care and Use Committee, The Johns Hopkins University (Baltimore, MD). Adult male mice (C57BL/6, 20–25 g; Jackson Laboratories, Bar Harbor, ME) were used in this study.

For immunofluorescent (IF) staining of nicotinic receptors, we used basic indirect IF, but for high-affinity receptor for NGF (TrkA) receptors, we used tyramide signal amplification. Mice were killed by an overdose of pentobarbital (75 mg/kg, intraperitoneal) and transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) containing heparin (100 U/ml) and procaine (0.2%), followed by fixative (4% formaldehyde in PBS), and postfixed for 2 hours (4°C). After rinsing in PBS (24 h, 4°C), 3-mm squares of dorsal trachealis muscle containing parasympathetic ganglia were cryoprotected in 18% sucrose in PBS (24 h, 4°C), frozen in optimum cutting temperature (OCT) mounting medium, and alternating serial transverse sections (10 μm) were collected on lysine-coated slides and air dried. To minimize nonspecific binding of the polyclonal secondary antibody (raised in donkey), blocking solution (PBS containing 10% donkey serum, 1% BSA, and 0.1% Tween 20) was applied for 1 hour at room temperature. For tyramide signal amplification, endogenous peroxidase activity was blocked (Dako, Carpinteria, CA; 30 min). The sections were then incubated overnight (4°C) in a combination of goat antibody recognizing the α3 nAChR subunit (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1:50), and rabbit antibody recognizing either the β4 nAChR subunit (Alomone Labs, Jerusalem, Israel; 1:100) or the N terminus of the TrkA receptor (Santa Cruz Biotechnology; 1:50) diluted in PBS containing 1% BSA and 0.5% Triton X-100. Species-specific Alexafluor-conjugated secondary antibodies (Invitrogen/Life Technologies, Carlsbad, CA) were used for labeling primary antibodies. For negative control, separate sections were processed similarly, but the primary antibody was replaced with rabbit or goat IgG to evaluate nonspecific staining; positive control was mouse superior cervical ganglia. After rinsing, a tyramide signal amplification kit (Invitrogen/Life Technologies) was used for TrkA; briefly, sections were incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin (1 μg/ml) and then with Alexafluor 594–conjugated tyramide in amplification solution. All slides were rinsed and coverslipped with ProLong Gold (Invitrogen/Life Technologies), viewed, and photographed with an epifluorescence microscope (Olympus BX50; Olympus America, Inc., Melville, NY).

### Electrophysiology Tissue Preparation and Recording

Mice were killed with an overdose of pentobarbital (75 mg/kg, intraperitoneal), exsanguinated, and transcardially perfused with Krebs bicarbonate buffer (60 ml, 20–22°C). The composition of this buffer was 136 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 1.2 mM $NaH_2PO_2$, 14.3 mM $NaHCO_3$, and 11 mM dextrose, equilibrated with 95% $O_2$/5% $CO_2$ (pH 7.4). Atropine (0.1 μM) was added to Krebs buffer for all electrophysiology experiments.

Intracellular (current clamp) recordings were similar to those previously reported (18): the trachea and main-stem bronchi were dissected, keeping the right vagus nerve intact and connected to the airways by the recurrent laryngeal and peribronchial nerves to maintain preganglionic innervation. The trachea and bronchus were then cut longitudinally along the ventral midline, opened, and tightly pinned as a sheet, with the dorsal surface of the tissue facing upwards, to Sylgard (Dow Corning Corp., Midland, MI), which lined the floor of a recording chamber. Unstained ganglia were exposed in the trachea and bronchus by fine dissection with the aid of a stereomicroscope (Olympus America, Inc., Melville, NY) at magnifications of 25–80×, using reflected transmitted light as previously described (18). Tracheal ganglia were located over the tracheal smooth muscle by following inlet nerves arising from the recurrent laryngeal nerves, and bronchial ganglia from the peribronchial nerves. The recording chamber was then transferred to the fixed stage of a compound microscope equipped with a long working-distance objective (20×; Olympus America). Krebs buffer (described previously here) was continuously superfused over the tissue, being introduced at one end of the chamber (250-μl volume) and suctioned away at the other. Using a suction electrode, the vagus nerve was pulled into a capillary tube containing, and surrounded by, chloridized silver wires, which were connected to a stimulator (Grass, Astro-Med, Inc., West Warwick, RI) for preganglionic nerve stimulation and generation of fEPSPs. Ganglia could then be visualized (at 200×) for impalement of neurons with

a microelectrode. The tissue was allowed to equilibrate for at least 1 hour in flowing (5–8 ml/min) oxygenated Krebs buffer at 36–37°C in the recording chamber before experimentation.

fEPSPs were elicited by 1-Hz square pulses delivered to the rostral end of the vagus nerve, 10–30 mm from the ganglion. For recording sub-threshold synaptic potentials, QX314 (3 μM; Tocris Bioscience, Ellisville, MI) was added to the electrode solution (3 M KCl); this compound is an intracellular sodium channel blocker that inhibits action potential generation, allowing accurate measurement of synaptic potentials. The amplitude of 100 consecutive vagus nerve–evoked fEPSPs was averaged in the presence of vehicle (as control) before drug application, and during and after application of NGF (β subunit of NGF, rat recombinant; 1, 10, 30, and 100 ng/ml). Vehicle controls were performed using PBS diluted up to 1,000-fold or 50% DMSO diluted 10,000-fold. Control active and synaptic membrane property measurements (no QX314 in the electrode solution) were recorded before NGF application for 2 minutes, and active membrane properties were determined upon cessation of exposure and at 5-minute intervals thereafter.

For voltage clamp recordings, tissue preparation was similar to that described previously here, except that isolated ganglia were pinned to the recording chamber and enzymatically treated (Hanks' balanced salt solution, $Ca^{++}$ and $Mg^{++}$ free with 2 mg/ml collagenase, 2 mg/ml dispase, 30 min, 37°C) before perfusion with oxygenated Krebs buffer (described previously here), and patch clamp recordings were performed after formation of a gigaohm seal (19). Neurons were voltage clamped at −50 mV and the amplitude and duration of the inward current evoked by application of the nicotinic receptor agonist, 1,1-dimethyl-4-phenylpiperazinium (DMPP; 1–100 μM, 10 s) was recorded before and during NGF, as described previously here. In all experiments, only one concentration of NGF was applied to a single neuron.

All reagents used to make Krebs buffer solution were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). K-252a was purchased from ICN Biochemicals, Inc. (Aurora, OH). All other reagents were purchased from Sigma-Aldrich Co. (St. Louis, MO). Stock solutions of DMPP (10 mM) and atropine (1 mM) were dissolved in water; NGF (β subunit, rat recombinant, 1.0 mg/ml) in PBS, indomethacin (100 mM) in ethanol, and K-252a (2 mM) in 50% DMSO.

### Statistical Analysis

All data are expressed as the arithmetic mean (±SEM). Unless otherwise stated, an *n* value indicates one neuron from one animal; thus, "*n* = 6" is six neurons from six different animals. Control values for fEPSP amplitude, resting membrane potential, membrane input resistance (Ri), cumulative after-hyperpolarizing potential (AHP) duration, and amplitude were noted before NGF application. These values were compared with peak changes evoked by NGF in the same cell using paired Student's *t* tests. Statistical tests were performed using the Prism statistics program (GraphPad Software, Inc., San Diego, CA). Statistical significance was accepted at the 0.05 level of probability (*P*).

## Results

### TrkA and nAChR IF Staining

Eleven ganglia (from six mice) containing 6–31 neurons (18.5 ± 0.9 neurons/ganglion; *n* = 171 neurons) were located on the serosal surface from six mouse tracheas. These ganglia were in a similar location as ganglia in mouse tracheas containing neurons reported to be cholinergic, based on their expression of choline acetyltransferase (20). Based on IF staining, sections of mouse airway parasympathetic ganglia show neurons that expressed nAChRs containing the α3 (Figures 1A and 1D) and β4 subunits (Figure 1B). In different sections of tracheal ganglia, all neurons (73 in four ganglia, four mice) had varying levels of TrkA immunoreactivity (Figure 1C). Neurons in sections of the same ganglia used for negative controls (no specific primary antibody) had no or sparse staining dispersed outside of the neuronal cell bodies (data not shown).

### Electrophysiological Recording

Intracellular recordings of passive and active membrane properties were made from the cell bodies of neurons located in tracheal and bronchial parasympathetic ganglia. Consistent with previous studies of mouse airway parasympathetic neurons (18, 21), resting membrane potential and membrane resistance averaged −50 (±5) mV and 63 (±7) MΩ (*n* = 20), respectively, and did not change significantly during the course of experimentation or in the presence of,

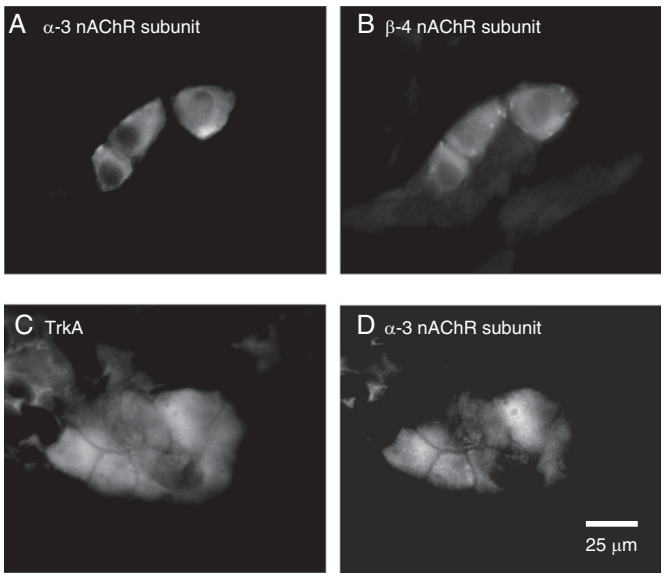


**Figure 1.** Nicotinic acetylcholine (ACh) receptors (nAChRs) and tropomyosin-related kinase (Trk) A receptors on mouse airway parasympathetic ganglionic neurons. (*A*) The α-3 nAChR subunit immunofluorescence is on three airway parasympathetic neurons. (*B*) The same section as *A*, showing that the same three neurons also express the β-4 nAChR subunit; the puncta are, most likely, areas of synaptic organization. (*C*) Neurons in an airway ganglion show TrkA immunofluorescence. (*D*) The same neurons in the same section as *C* express the α-3 nAChR subunit. *Scale bar* in *D* is for all images.

or after, NGF exposure (1–100 ng/ml; $n = 4$–6). As previously reported (18), the majority (63%) of mouse airway ganglioic neurons respond to a prolonged depolarizing pulse with a burst of action potentials followed by accommodation (phasic neurons), and the remaining neurons fire action potentials throughout the pulse (tonic neurons [18]). There was no significant change in the accommodation properties (tonic and phasic action potential patterns) by these neurons during a 2-minute application of 30 or 100 ng/ml of NGF ($n = 5$) or during 30-minute exposure ($n = 4$). NGF (30 ng/ml, 2 min) decreased the action potential after-hyperpolarization amplitude and duration after a single action potential by 7 (±4) mV and 28 (±10) ms, respectively ($n = 8$; $P < 0.05$; Figure 2). After four action potentials (cumulative AHP), there was no significant decrease in cumulative AHP amplitude (12 ± 7 mV control, 9 ± 3 ms after 100 ng/ml NGF, 2 min; $n = 8$), but the duration decreased from 259 (±23) ms (control) to 178 (±36) ms after NGF-β (100 ng/ml), a reduction of 31% ($n = 6$; $P < 0.05$; data not shown). This was not due to cyclo-oxygenase products as previously reported (22), because indomethacin was present. NT-3 (100 ng/ml; $n = 4$), or BDNF (100 ng/ml; $n = 4$) had no effect on active or passive electrophysiological properties (data not shown); at this concentration, these NTs would most likely activate both low (p75NTR) and high-affinity (Trk) receptors, but, as this concentration had no effect, we did not investigate the effects of these NTs at lower concentrations.

To determine whether NGF affected synaptic transmission, fEPSPs were evoked by vagus nerve stimulation and amplitudes measured with an intracellular electrode before and after a 2-minute exposure to NGF. In control neurons, fEPSPs that were sub-threshold for action potential formation had an amplitude of 16.2 (±1.1) mV ($n = 6$; Figure 3A). After exposure to NGF (10 ng/ml, 2 min), the amplitude of fEPSPs was increased to 21.8 (±2.8) mV in all neurons ($n = 6$, $P < 0.05$; Figure 3B), as was the area of the fEPSPs (from 23.3 ± 9.2 Vm · ms to 43.0 + 4.1 Vm · ms; $P < 0.05$; Figure 3D). A higher (30 ng/ml, $n = 5$) concentration of NGF also potentiated fEPSPs by 27%, but the lowest concentration tested (1 ng/ml; $n = 4$) had no effect (Figure 3C). The potentiation of fEPSP amplitude (NGF at 10 ng/ml) lasted the entire length of the recording (up to 37 min, $n = 4$). Indomethacin (30 μM) had no effect on the potentiation of fEPSP amplitude evoked by two different concentrations of NGF (30 ng/ml, $n = 3$; 100 ng/ml, $n = 3$; data not shown). NT-3 (100 ng/ml, $n = 4$) and BDNF (100 ng/ml, $n = 4$) had no effect on fEPSP amplitude (data not shown); for reasons stated previously here, we did not investigate the effects of these NTs at lower concentrations.

To determine if NGF was directly affecting nicotinic receptor channels, the inward current evoked by the nicotinic agonist, DMPP (1, 5, and 10 μM), was also assessed before and after administration of NGF. The inward current evoked by 1 μM DMPP (−179 ± 27 pA; Figure 4A) was increased by 10 ng/ml NGF (−224 ± 21 pA, $n = 4$; Figure 4B). A concentration of 30 ng/ml NGF potentiated DMPP responsiveness to a similar level ($n = 4$; Figure 4C), and the lowest concentration of NGF tested (1 ng/ml) had no effect on the DMPP response (Figure 4C). NGF (30 and 100 ng/ml) had no effect on larger DMPP responses evoked by 5 and 10 μM

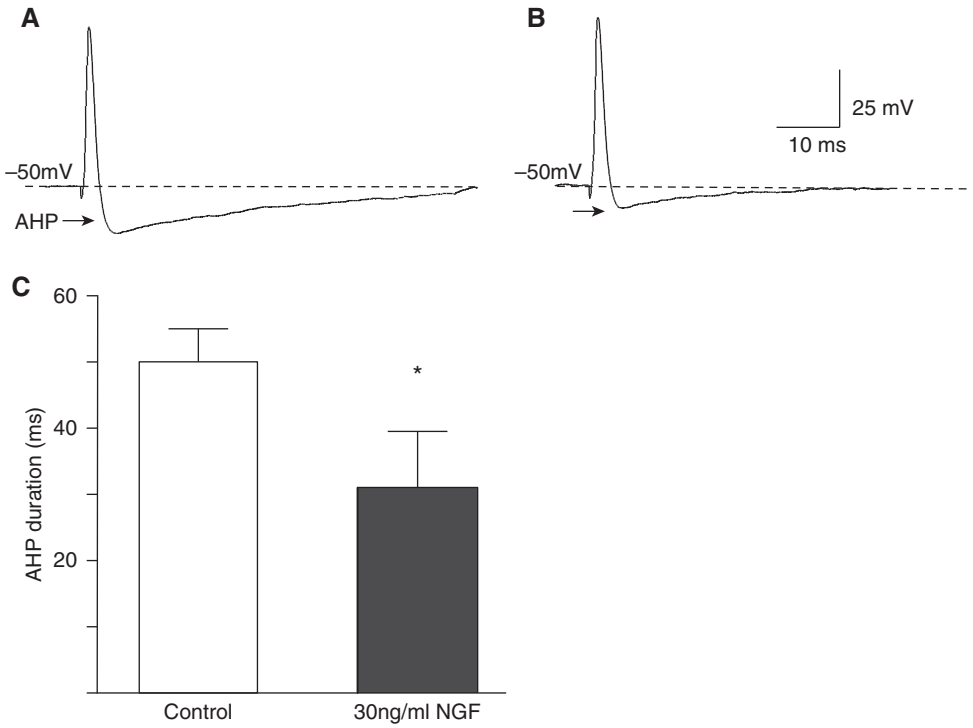


**Figure 2.** Nerve growth factor (NGF) decreases the action potential after-hyperpolarization amplitude and duration in a mouse airway parasympathetic neuron. (*A*) A control action potential after-hyperpolarizing potential (AHP; *arrow*) is 52 ms long with an amplitude of 17 mV. (*B*) In the presence of NGF (30 ng/ml), the AHP (*arrow*) is 34 ms, 11 mV. *Scale bar* in *B* is for both traces. (*C*) Summary of effects of NGF (30 ng/ml) on AHP duration ($n = 6$; *$P < 0.05$ as compared with control). Data are expressed as the arithmetic mean (±SEM).

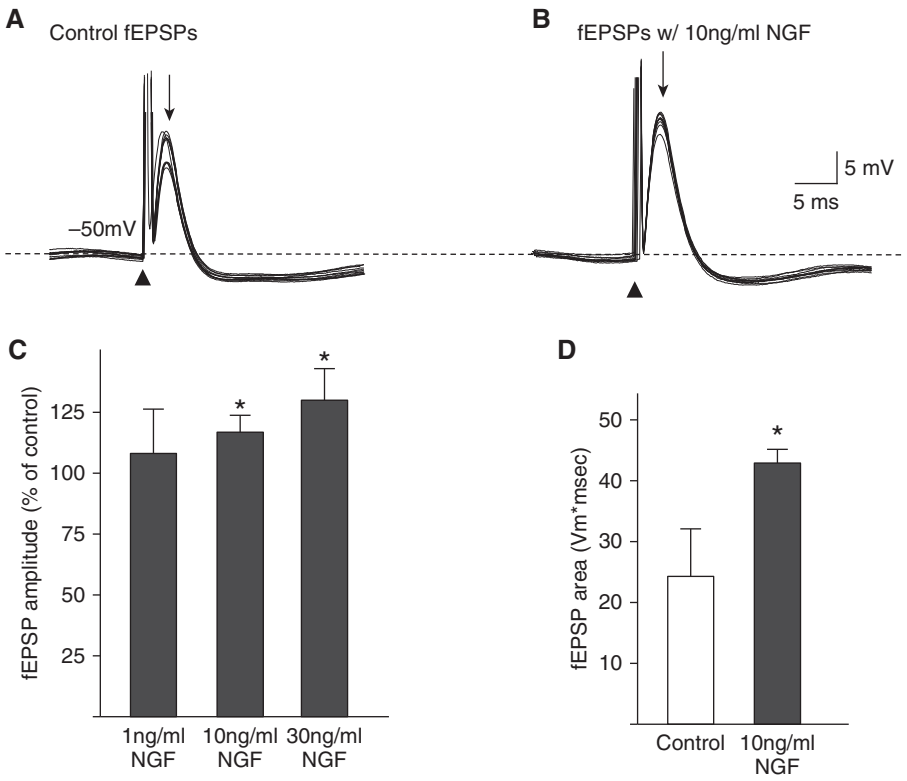


**Figure 3.** NGF increases fast excitatory postsynaptic potentials (fEPSPs) in a mouse ganglionic neuron. (*A*) Ten consecutive superimposed traces of control fEPSPs (18 ± 0.7 mV, measured at *downward arrow*), were evoked at 1 Hz stimulation frequency (10 V, 0.1 ms pulse duration, at *arrowhead* shock artifact). (*B*) Ten consecutive fEPSPs were then evoked with the same stimulus (*arrowhead*) in the same cell in the presence of NGF (30 ng/ml), which increased the amplitude of the fEPSPs to 21 (±0.1) mV (*downward arrow*). *Scale bars* in *B* are for all traces. (*C*) Summary of effects of NGF on fEPSPs ($n = 4$–5; $^*P < 0.05$ as compared with control). Data are expressed as the arithmetic mean (±SEM).

DMPP ($n = 4$ each; data not shown). To examine whether the effects of NGF were mediated by TrkA receptor–associated tyrosine kinases, nicotinic responsiveness to DMPP was measured before and after NGF in the presence of the tyrosine kinase inhibitor, K-252a. K-252a (200 nM) blocked the NGF-induced increase in DMPP responsiveness (10 μM; Figure 4C). NT-3 (100 ng/ml, $n = 4$) and BDNF (100 ng/ml, $n = 4$) had no effect on inward currents evoked by nicotinic receptor stimulation (data not shown).

## Discussion

In this study, we examined the direct effects of NGF on membrane properties of mouse lower airway parasympathetic ganglionic neurons in adult mouse lower airways. These effects are most likely due to activation of specific receptors for NGF and, based on IF staining, mouse airway cholinergic parasympathetic ganglionic neurons express TrkA receptors, the high-affinity receptor for NGF, not unlike what is observed for all cholinergic neurons in the CNS (23) and peripheral nervous system (11). This is the first study to show that airway parasympathetic neurons express nAChRs containing the α3 and β4 subunits, which may be the primary nAChR subtypes associated with synaptic transmission in this cell type, similar to reports on other autonomic neurons (3). We provided evidence that the effects of NGF include potentiation of synaptic transmission, most likely by increased nAChR activity.

Long-term exposure to NTs has effects on single-channel properties that can alter active membrane properties (e.g., Ref. 24), but only recently have the acute effects of NTs on electrophysiological properties been examined, and these are usually on neonatal or neurons in culture (e.g., Refs. 25, 26). In our study, NGF had immediate effects on the AHP after a single action potential or a volley of action potentials, but did not affect other measured active (action potential) or resting potential and resistance (passive membrane properties). The action potential AHP duration decrease caused by NGF may be due to a decrease in calcium ion current (27), or a direct effect on potassium and possibly chloride channels. Unlike mouse sensory neurons (26), action potential duration was unaffected by NGF and, thus, alteration of calcium-sensitive AHP is unlikely (27). The NGF-induced decrease in AHP is comparable to that reported for parasympathetic neurons on the guinea pig bronchus, where the cumulative AHP was reduced by prostaglandin (PG) E2, but

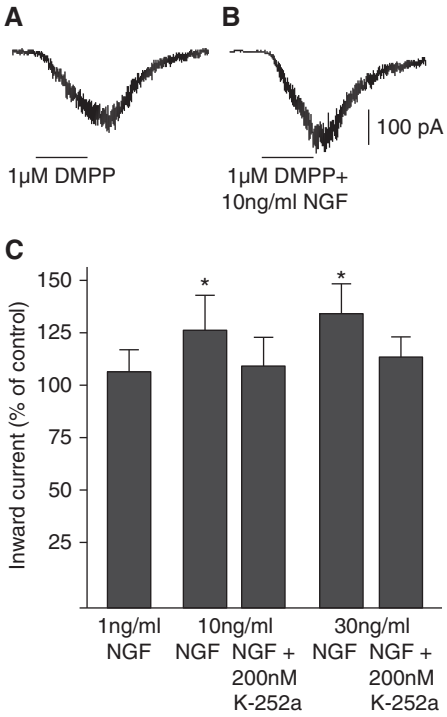


**Figure 4.** Patch clamp recording (whole cell, voltage clamp mode) of (*A*) a control 1,1-dimethyl-4-phenylpiperazinium (DMPP)–induced (1 μM, 30 s, *horizontal bar*) inward current in a mouse airway ganglionic neuron. (*B*) A trace showing the effect of NGF (10 ng/ml) pretreatment on the same exposure to DMPP. The neuron was clamped at −50 mV. *Vertical calibration bar* in *B* is for both traces. (*C*) Summary of the effect of NGF on DMPP–induced (1 μM, 30 s) inward currents and how K-252a (200 nM) blocks these effects ($n = 4$–6; $^*P < 0.05$ as compared with control). Data are expressed as the arithmetic mean (±SEM).

PGE2 did no alter the phasic neuron action potential pattern or the single action potential duration (22); however, it is unlikely that cyclo-oxygenase products, such as PGs, were involved, because indomethacin was present in the perfusion buffer (22). Accommodation by phasic neurons in guinea pig bronchial ganglia is not coupled to potassium currents that are calcium-activated potassium currents (27). During the AHP, it is more difficult to generate the next action or depolarizing synaptic potential: that NGF reduced the AHP duration may indicate increased excitability, as observed for cholinergic neurons in the CNS (28), and thus may allow preganglionic input to reach threshold for action potential generation (1).

Synaptic transmission involves preganglionic activation of nicotinic receptors on postganglionic neurons, generating an fEPSP that reaches threshold for action potential generation. The likelihood of whether an action potential is generated is dependent on many factors, such as: whether a sufficient level of ACh is released from preganglionic terminals; the number and location of postganglionic nicotinic synapses; as well as the activity state of the postganglionic neuron (1). We attempted to determine if the effects of NGF on synaptic transmission was due to interactions with ionotropic nAChR located on postganglionic neurons. We observed that all but the lowest concentration of NGF increased nicotinic agonist–induced inward current by the ganglionic neurons, a very definitive indication that nAChRs are directly affected by NGF, as has been reported for TrkB receptors in CNS neurons (29). The effects of NGF were inhibited by K-252a, a tyrosine kinase inhibitor; this may reveal an intracellular interaction between the TrkA receptors, which have tyrosine kinase activity, and the nAChR cation channel. Several methods were used to eliminate the possibility of activation of p75 receptors and/or other specific Trk receptors: at a concentration of 10 ng/ml, NGF should only activate TrkA receptors, and not p75 receptors (4), and that, at the concentrations of BDNF and NT-3 used, these did not affect the nAChR response, indicate that TrkB and TrkC receptors, respectively, as well as p75 receptors, are not involved. It should also be noted that the increased nicotinic responses were caused by a low concentration of NGF (10 ng/ml), which can activate TrkA receptors, but not other Trk receptors or the p75 receptors. BDNF or NT-3, at the concentrations tested, should stimulate specific TrkB and TrkC receptors, respectively, as well as p75 receptors (4). One possible mechanism for the increased nicotinic response by NGF may be an augmented chance of opening nAChRs, which increases nicotinic depolarization by neurons in airway ganglia, similar to the effect of NGF to increased nicotinic agonist–induced rubidium ion influx in pheochromocytoma 12 cells (25). Increase in responsiveness to nicotinic agonists may underlie the observed NGF-induced increase in fEPSP amplitude by neurons in airway ganglia.

Several studies have shown that disease-induced alterations in lung function are associated with elevated NT levels. Smooth muscle cells and epithelium are possible sources of NGF in an allergic mouse model of asthma, in addition to infiltrating cells, such as macrophages, eosinophils, and mononuclear cells (reviewed in Ref. 8). Based on the distances between the epithelium and adventitia, NGF released from any of these cell types may diffuse to parasympathetic nerves. Neurons in airway parasympathetic ganglia have several types of acute or rapid synaptic plasticity that may increase probability of generating a postsynaptic action potential, which may affect how ganglionic neurons integrate CNS signals and, thus, affect autonomic regulation of airway smooth muscle tone. In this study, we provide evidence that nicotinic responses by airway ganglionic neurons are altered by NGF, exclusively at postsynaptic sites. Our results may provide a new role for this NT in affecting parasympathetic function in the bronchi. Given the possibility that NGF is elevated in airway diseases, these results demonstrate that NGF effects on parasympathetic nerves may regulate airway smooth muscle and, thus, airway caliber. Furthermore, these studies were done in mice, which are routinely used to study airway diseases, such as asthma (30), where contraction of airway smooth muscle induced by allergen has a well-defined parasympathetic cholinergic component (21, 31). Thus, the cholinergic tone could be amplified if the synaptic efficacy is increased due to higher levels of NGF. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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