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Research Report

Effects of acetylcholine and electrical stimulation on glial cell line-derived neurotrophic factor production in skeletal muscle cells

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ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophic factor required for survival of neurons in the central and peripheral nervous system. Specifically, GDNF has been characterized as a survival factor for spinal motor neurons. GDNF is synthesized and secreted by neuronal target tissues, including skeletal muscle in the peripheral nervous system; however, the mechanisms by which GDNF is synthesized and released by skeletal muscle are not fully understood. Previous results suggested that cholinergic neurons regulate secretion of GDNF by skeletal muscle. In the current study, GDNF production by skeletal muscle myotubes following treatment with acetylcholine was examined. Acetylcholine receptors on myotubes were identified with labeled alpha-bungarotoxin and were blocked using unlabeled alpha-bungarotoxin. The question of whether electrical stimulation has a similar effect to that of acetylcholine was also investigated. Cells were stimulated with voltage pulses; at 1 and 5 Hz frequencies for times ranging from 30 min to 48 h. GDNF content in myotubes and GDNF in conditioned culture medium were quantified by enzyme-linked immunosorbant assay. Results suggest that acetylcholine and short-term electrical stimulation reduce GDNF secretion, while treatment with carbachol or long-term electrical stimulation enhances GDNF production by skeletal muscle.

1. Introduction

Glial cell line-derived neurotrophic factor (GDNF) was first purified by Lin et al. (1993) as a survival factor for dopaminergic neurons. GDNF is widely distributed in neuronal and non-neuronal tissues (Springer et al., 1995). GDNF exerts its survival effects on other subpopulations of neurons in the central and peripheral nervous systems (Henderson et al., 1994; Moore

Abbreviations: ACh, acetylcholine; AChRs, acetylcholine receptor; αBTX, alpha-bungarotoxin; ATCC, American Type Culture Collection; BSA, bovine serum albumin; CCh, carbachol; CPRG, chlorophenol red-β-D galactopyranoside; DMEM, Dulbecco's Modified Eagle's Medium; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GDNF, glial cell line-derived neurotrophic factor; PBS, Phosphate buffer saline; TTX, tetrodotoxin

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et al., 1996; Trupp et al., 1995). Specifically, GDNF is characterized as a survival factor for spinal motor neurons (Henderson et al., 1994). The trophic factor is synthesized and released by skeletal muscle, and acts as a muscle-derived neurotrophic factor for spinal motor neurons (Suzuki et al., 1998a).

During development, GDNF rescues motor neurons from programmed cell death (Oppenheim et al., 1995), acts as a chemoattractant, and assists with motor axonal guidance to motor neuron target tissues (Dudanova et al., 2010; Kramer et al., 2006). GDNF facilitates synaptic transmission (Wang et al., 2001), maintains synaptic activity (Zwick et al., 2001), plays a role in enhancing nerve recovery after injury (Cote et al., 2011; Dupont-Versteegden et al., 2004; Hashimoto et al., 2005; Houenou et al., 1996; Naveilhan et al., 1997; Oppenheim et al., 1995; Zhang et al., 2009) and muscle overexpressing GDNF displays hyperinnervation of endplates (Nguyen et al., 1998). These findings support the hypothesis that motor neurons depend on GDNF as a target-derived neurotrophic factor and GDNF secreted by skeletal muscle may be important for motor neuron survival (Angka et al., 2008; Bohn, 2004).

Although much is known about the effects of GDNF on motor neurons, little is known about factors regulating GDNF synthesis and release by skeletal muscle. Denervation of skeletal muscle causes an increase in GDNF expression (Suzuki et al., 1998b; Lie and Weis, 1998), while muscle cells co-cultured with neural cells in vitro secrete less GDNF (Vianney and Spitsbergen, 2011). These findings suggest that the innervation status of skeletal muscles plays a role in regulating the amount of GDNF produced by muscle. In cell culture, the ratio of GDNF inside skeletal muscle is higher than that released into culture medium (Vianney and Spitsbergen, 2011), suggesting that GDNF may be synthesized and stored in a manner similar to neurotrophins (Poo, 2001). In vivo studies have shown that GDNF in skeletal muscle can be regulated in an activity-dependent manner, such as with physical exercise (McCullough et al., 2011; Wehrwein et al., 2002).

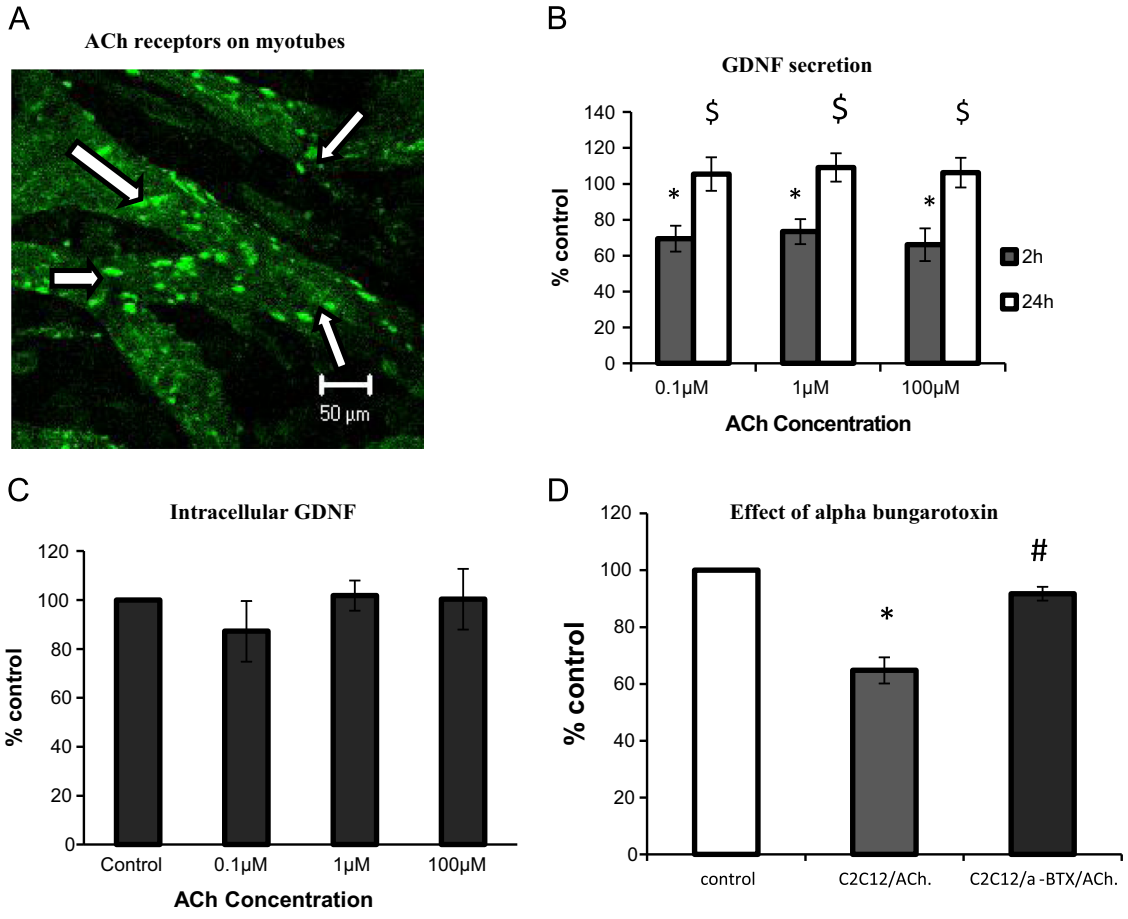

Fig. 1 – Effect of ACh on GDNF production by skeletal muscle cells. Myoblast cells were grown and allowed to differentiate into myotubes. A. AChRs on myotubes (white arrows). Culture medium containing α-BTX (200 nM) conjugated to AlexaFluor 488 (green) was added to myotubes and incubated for 1 h in a standard incubator. Following 1 h of treatment cells were fixed with 4% paraformaldehyde and viewed on a confocal microscope. B–C, 7-day-old myotubes were treated with culture medium containing ACh at concentrations of 0.1 µM, 1 µM, and 100 µM. Conditioned culture medium and cells were collected at 2 h and 24 h. B. ACh inhibits GDNF secretion following 2 h but not 24 h of exposure. C. ACh had no effect on intracellular GDNF content. D. Blocking AChRs with α-BTX prevented the effects of ACh on GDNF secretion at 2 h. An asterisk (*) indicates a significant decrease from control, dollar sign ($) indicates a significant difference in GDNF levels between samples collected at 2 h and that collected after 24 h, pound sign (#) indicates a significant difference in GDNF levels between cells treated with or without α-BTX. Values are presented as means ± S.E.M, $P \leq 0.05$.