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Passive stretching decreases glial cell line-derived neurotrophic factor expression in skeletal muscle and is dependent upon acetylcholine receptor activation.



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Abstract

Motor neurons receive trophic support from the tissues they innervate. One molecule that is important for peripheral motor neurons is glial cell-line derived neurotrophic factor (GDNF). We have previously reported that GDNF is regulated in an activity-dependent manner in skeletal muscle. For this study we examined the short-term effects of passive stretching on the expression of GDNF in skeletal muscle. Extensor digitorum longus (EDL) muscles were removed from 4 week old Sprague Dawley rats and placed in oxygenated tissue baths containing Ringer's solution. Tissues were passively stretched for 4 hours while their contralateral counterparts remained in baths at resting tension. We found that GDNF protein content significantly decreased after 4 hours of passive stretching. Muscles pre-treated with the acetylcholine receptor antagonist alpha-bungarotoxin and subsequently subjected to passive stretching displayed unaltered GDNF expression. Alpha-bungarotoxin treatment alone had no significant effect on GDNF levels in skeletal muscle in the absence of any stretching. These results indicate that short-term passive stretching decreases GDNF expression and that the effect is mediated through acetylcholine receptor activation.

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Introduction

Neurotrophic factors are an important part of the communication that takes place at the neuromuscular junction (NMJ). They act as extracellular messaging proteins that bind to receptors on motor neurons and signal local and cellular changes. One such neurotrophic factor is glial cell line-derived neurotrophic factor or GDNF. GDNF was first discovered in the brain (Lin *et al.* 1993) but further investigation determined that GDNF is critical for proper motor neuron development (Henderson *et al.* 1994). Over expression of GDNF in skeletal muscle of transgenic mice results in hyperinnervation of the NMJ during development (Zwick *et al.* 2001) demonstrating its ability to keep neurons alive during a time when they are developmentally removed. It has recently been determined that GDNF is important for motor neuron innervation of muscle spindle fibers – the fibers responsible for determining stretch and position of skeletal muscles (Whitehead *et al.* 2005).

A recent study in our lab demonstrated that GDNF expression is regulated in an activity-dependent manner in rat skeletal muscle (Wehrwein *et al.* 2002). We determined that rats exercised by run training had an increase in GDNF protein expression in active hind limb muscles. One of the physiological forces acting upon these muscles that could be responsible for altering GDNF protein content is stretch. It is the purpose of this study to identify the effects of short-term, passive stretching on GDNF protein content in skeletal muscle.

Aims

- Does passive stretching affect GDNF protein levels in skeletal muscle?
- Is acetylcholine receptor activation involved in this response?

Results

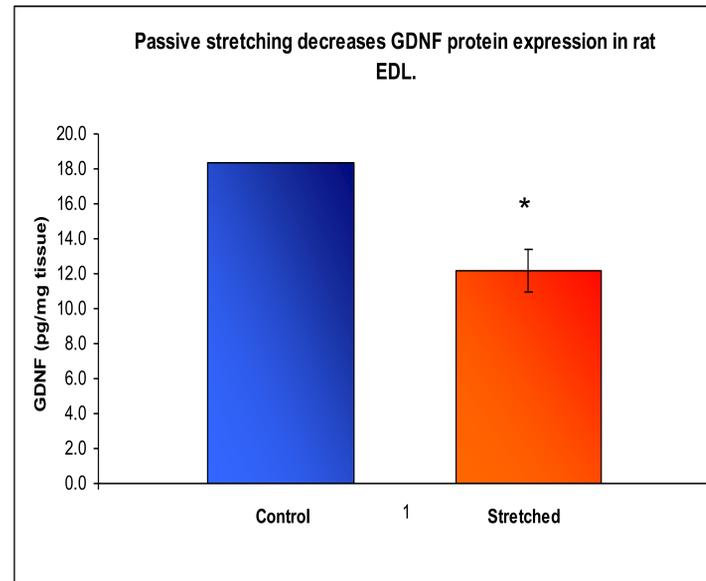


Figure 1. Summary data (n = 6) of GDNF protein content measured via ELISA from control and passively stretched EDL muscles. GDNF is significantly decreased in the stretched as compared to the sedentary group. Data represent the mean ± SE (p ≤ 0.05).

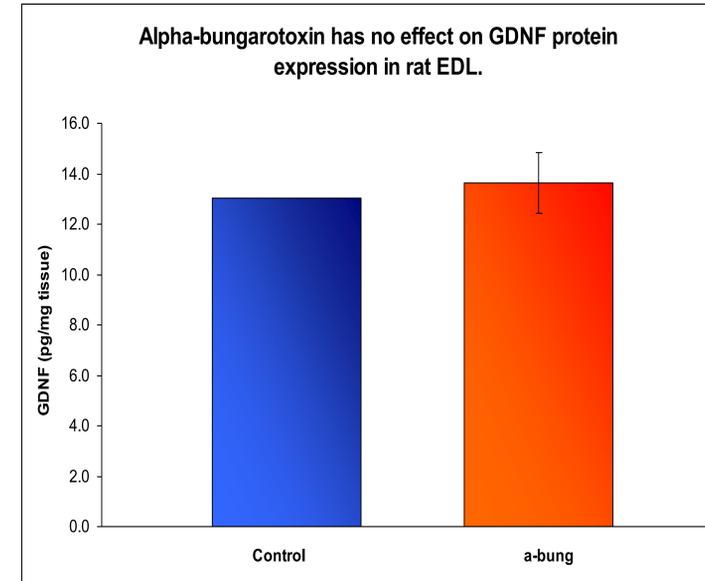


Figure 2. Summary data (n = 6) of GDNF protein content measured via ELISA from control and alpha-bungarotoxin treated EDL muscles. There is no significant difference between groups. Data represent the mean ± SE.

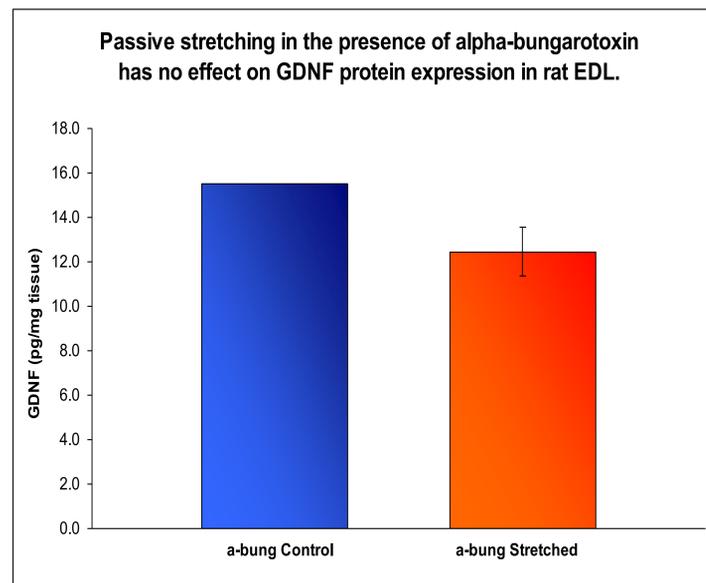


Figure 3. Summary data (n = 6) of GDNF protein content measured via ELISA from control and passively stretched EDL muscles pre-treated with alpha-bungarotoxin. There is no significant difference between groups. Data represent the mean ± SE.

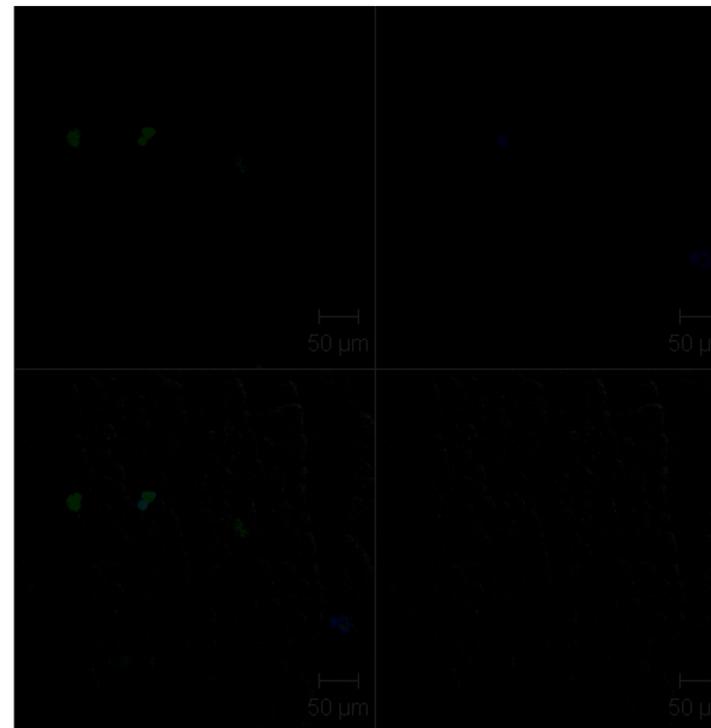


Figure 4. Top Left: Skeletal muscle section labeled with anti-GDNF antibody. Top Right: Immuno-positive staining for s46 antibody. Bottom Left: Merged image of all panels. Bottom Right: Transmitted light.

Methods

Test Subjects:

4 week-old male Sasco Sprague Dawley rats (Charles River Co.).

Bath Studies:

Paired hind limb muscles (extensor digitorum longus [EDL]) were removed and placed in tissue baths containing oxygenated Krebs' s Ringer solution. One side was chosen at random to be in the unmanipulated control bath while the contralateral tissue was mechanically stretched. The protocol for stretching frequency and duration was adapted directly from a field-stimulation protocol. The range of stretch was ± 15%. This is within the physiological range of stretch (10-15%) as determined for skeletal muscle (Chen and Grinnell 1997).

Determination of GDNF protein content:

Skeletal muscles were frozen on dry ice and stored at -80°C. The tissues were processed following manufacturers protocol for analysis of GDNF protein content using an enzyme-linked immunosorbant assay (ELISA) (R&D Systems, Minneapolis, MN). GDNF values were quantified and expressed as pg/mg of tissue weight.

Immunohistochemistry:

EDL were removed and fixed in Zamboni's fixative for 15 minutes. Tissues were then washed 3x5 minutes in phosphate buffered saline (PBS). Muscles were frozen in 2-methylbutane, embedded in mounting media and thaw-mounted to slides at 60 μm thick sections. Samples were then washed 3x5 min. in PBS and primary antibodies were added at 2ug/mL in 1% bovine serum albumin and 1% normal donkey serum in PBS with 0.3% Triton x-100 for 4 days. Samples were washed again 3x5 min. in PBS and secondary antibodies (donkey anti-rabbit conjugated to Alexa Fluor 488 and donkey anti-mouse 647) were added at 2ug/mL in PBS for 30 minutes. Samples were then placed on slides in a mixture of 50% PBS with 50% glycerol and sealed with a coverslip for visualization using a laser confocal microscope.

Statistical Analysis:

Data are represented as the mean ± SE. Data were analyzed using student's t-test assuming equal variances, or when required, assuming unequal variances as determined by F-test for homogeneity. Significance is established as p ≤ 0.05.

Summary

- Passive stretching decreases GDNF protein content in rat EDL.
- Alpha-bungarotoxin alone has no effect on GDNF protein in rat EDL.
- Passive stretching in the presence of alpha-bungarotoxin has no significant effect on GDNF protein content in rat EDL.
- GDNF protein is co-expressed at intrafusal muscle fibers of the rat EDL.

Conclusions

These results demonstrate that passive stretching decreases GDNF protein content in rat EDL and that this effect is dependent upon AChR activation. Blocking AChR activity with alpha-bungarotoxin attenuates the effects of passive stretching in EDL and provides a possible target for therapeutic intervention.

Acknowledgements

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