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Cholinergic Neurons Regulate and Utilize GDNF Secreted by C2C12 Skeletal Muscle Cells in Culture

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**Cholinergic neurons regulate and utilize GDNF secreted by C2C12 skeletal muscle cells in culture**

John-M. Vianney and John M. Spitsbergen, Western Michigan University, Kalamazoo, MI 49008.

**Abstract**

GDNF (glial cell-line-derived neurotrophic factor) regulates survival of neurons in both central and peripheral nervous systems. This study investigated the role of skeletal muscle cells in regulating GDNF production. In our previous study, we demonstrated that skeletal muscle cells produce GDNF in culture and suggested that cholinergic neurons might modulate GDNF production. In the present study, we examine the role of GDNF production by skeletal muscle cells and the effects of blocking AChRs on GDNF protein in myotubes. Results showed that skeletal muscle cells secrete high levels of GDNF in culture and that blocking AChRs with alpha-bungarotoxin (BTX) resulted in a decrease in GDNF secretion. These observations suggest that skeletal muscle cells do not secrete high levels of GDNF in muscle tissue due to the presence of high levels of AChRs.

**Introduction**

GDNF is a potent survival factor for populations of neurons in both central and peripheral nervous systems (Lin et al., 1993). The presence of GDNF in skeletal muscle cells has been reported, but its function in regulating GDNF production is unclear. In our previous study, we showed that skeletal muscle cells produce GDNF in culture and suggested that cholinergic neurons might modulate GDNF production. In the present study, we examined the role of GDNF production by skeletal muscle cells and the effects of blocking AChRs on GDNF protein in myotubes.

**Aims**

- **a.** Examine GDNF production by skeletal muscle cells in culture.
- **b.** Localize GDNF in skeletal muscle and at the nerve-muscle contact.
- **c.** Examine the role that nerve cells play in regulating GDNF production by skeletal muscle.
- **d.** Examine if cholinergic nerve cells induce their effect through acetylcholine receptors.

**Results**

- **Figure 1.** GDNF protein production in myoblasts, myotubes, and NG108-15 cells. Extracts of 8-day-old myoblasts or 8-day-old myotubes or adult muscle were run on 18% SDS-PAGE gels and transferred to nitrocellulose membranes. Western blotting analysis was performed using a mouse anti-human GDNF antibody. The content of GDNF was quantified by ELISA. Figure 2 shows the results of Western blotting analysis.

**Neural cells reduce GDNF content by skeletal muscle cells in culture**

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<th>Treatment</th>
<th>GDNF content in myoblasts</th>
<th>GDNF content in myotubes</th>
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<td>Control</td>
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<td>BTX</td>
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<td>AChR antagonist</td>
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**Neural cells reduce GDNF content in myocytes**

**Neural cells reverse the action of neural cells on GDNF secretion**

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**Blocking AChRs reversed the action of neural cells on GDNF production in myoblasts**

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**Blocking AChRs did not reverse the action of neural cells on GDNF production in myotubes**

**Summary**

- C2C12 skeletal muscle cells produce and secrete GDNF in culture medium. Moreover, GDNF is secreted in cells isolated from skeletal muscle.
- NG108-15 neuronal cells regulate the production of GDNF by C2C12 skeletal muscle cells and may block the effects of GDNF on neuronal cells.
- There are no reports of GDNF production by skeletal muscle cells in culture, and the role of GDNF in the regulation of this production is unclear.

**Conclusions**

- Thanks to the Biological Sciences Department at Western Michigan University for providing essential resources to complete this project.
- This study suggests that neural cells regulate their own supply of GDNF produced by skeletal muscle, in part, via acetylcholine receptor activation.

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**References**