Electrical stimulation has opposing effects on glial cell line-derived neurotrophic factor expression in voluntary and involuntary muscle

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Abstract

Glia cell line-derived neurotrophic factor (GDNF) is a potent survival factor for sub-populations of neurons including somatic and autonomic motor neurons. These neurons depend, in part, on GDNF that is synthesized and secreted by their target tissues. Whether the processes regulating GDNF production in these tissues is similar or different is poorly understood. The current study compares the regulation of production of GDNF in skeletal muscle and cardiac muscle following electrical and chemical stimulation. We show that electrical stimulation has opposing effects on GDNF production in cardiac and skeletal muscle, where GDNF levels increase with long-term electrical stimulation in skeletal muscle and decrease with the long term electrical stimulation in cardiac muscle. An increase in GDNF levels from 13% to 128% of control was observed in skeletal muscle following 1 and 5Hz stimulation, respectively, whereas in cardiac cells, there was a rapid reduction in GDNF levels in cells that were stimulated at 5Hz. Conversely, treatment with acetylcholine leads to inhibition of GDNF production in both cardiac and skeletal muscle cells. Treatments of 1µM acetylcholine reduced GDNF secretion by 27% and 66% in skeletal muscle and cardiac muscle, respectively. Treatment with 100 µM acetylcholine caused a decline in GDNF levels by 34% and 91% in skeletal muscle and cardiac muscle, respectively. The current results suggest that GDNF expression may be differentially regulated in the heart and skeletal muscle. Understanding the regulation of GDNF production in these target tissues will provide a better understanding of how these processes may be modulated therapeutically.

Introduction

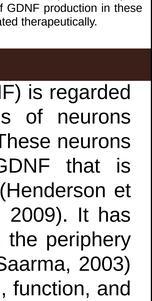
Glial cell line-derived neurotrophic factor (GDNF) is regarded as a potent survival factor for sub-populations of neurons including somatic and autonomic motor neurons. These neurons have been shown to depend, in part, on GDNF that is synthesized and secreted by their target tissues (Henderson et al., 1994; Martinelli et al., 2002; Shneider et al., 2009). It has been shown that a number of different tissues in the periphery express GDNF (Moore et al., 1996; Sariola and Saarma, 2003) and these target tissues differ in their composition, function, and in the case of different muscle cell types, their contractile characteristics. Whether the processes regulating GDNF production in these different tissues is similar or different is poorly understood.

GDNF protein production in skeletal muscle has been shown to be altered in vivo following exercise and in vitro following electrical field stimulation (McCullough et al., 2011). The goal of the current studies is to determine whether GDNF production by voluntary and involuntary muscle cells is regulated by electrical activity.

Study Aims

- To examine the effect of electrical stimulation on GDNF production in voluntary and involuntary muscle cells.
- To determine whether acetylcholine and electrical stimulation exert similar effect on GDNF production in voluntary and involuntary muscle cells.

Material and Methods



1. Cell culture: Figure 1A & B show differentiated cardiac (HL-1) cells and skeletal myotubes (C2C12) in culture. Cells were grown and maintained in a water saturated incubator using 95% air and 5% CO_2 during all electrical and chemical treatments. Samples of conditioned medium and cells were collected between 0h, and 24hours following treatment.

2. Electrical stimulation: Figure 1C shows a 6-well plate with electrodes. Cells were stimulated directly using stainless steel wire electrodes. For each experiment three wells were stimulated and three other wells served as controls. Cells were stimulated at 1Hz or 5Hz with an approximate 24V 30ms pulse for durations between 30min and 48h. Pulses were generated by a Grass S88 stimulator and applied via a custom-made voltage buffer circuit. The buffer circuit was capacitively coupled to the electrodes to reduce electrolysis effects.

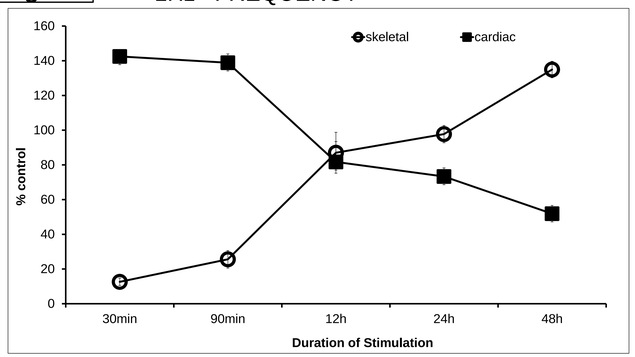
3. Chemical treatments: Cells were treated with fresh medium containing 1µM and 100µM ACh. To block voltage-gated sodium channels on skeletal myotubes, cells were pre-treated with 100uM Tetrodotoxin (TTX). Samples of conditioned culture medium and harvested cells were collected at 0h, 2h, and 24h.

4. GDNF protein analysis: GDNF protein content was examined by enzyme-linked immunosorbant assay (ELISA).

Results

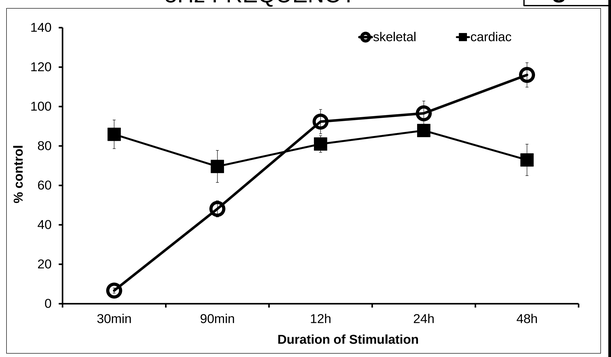
Electrical stimulation has opposite effects on GDNF secretion by skeletal and cardiac muscle cells

Fig. 2A



A. Effect of 1 Hz electrical stimulation (ES) on GDNF secretion by skeletal myotubes versus cardiac muscle cells. Cells were plated on 6-well plates and allowed to differentiate. Cells were stimulated at 1Hz frequency. Short-term ES (30min-90min) inhibited GDNF secretion in myotubes while increasing GDNF in cardiac muscle cells. The excitatory or inhibitory effect was reduced as the duration of ES was increased. GDNF protein content was examined by ELISA. Values are presented as mean ± S.E.M. (P≤ 0.05).

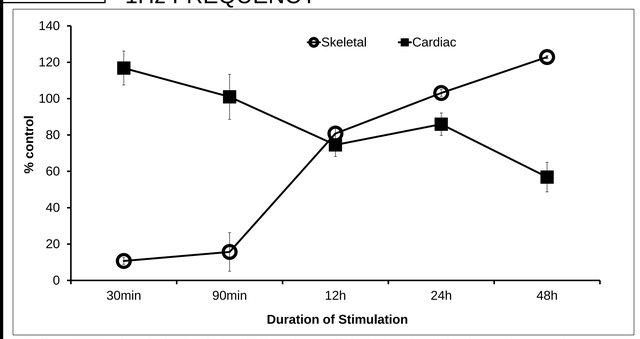
Fig.2B



B. Effect of 5Hz electrical stimulation on GDNF secretion by skeletal muscle myotubes and cardiac muscle cells. Cells were electrically stimulated at 5Hz. The inhibitory effect was reduced as the duration of ES increased in skeletal muscle, the effect persisted up to 24 hours of stimulation. Unlike the skeletal myotubes, the 5Hz had only an inhibitory effect in cardiac muscle cells. GDNF protein content was determined by ELISA. Values are presented as mean ± S.E.M. (P≤ 0.05).

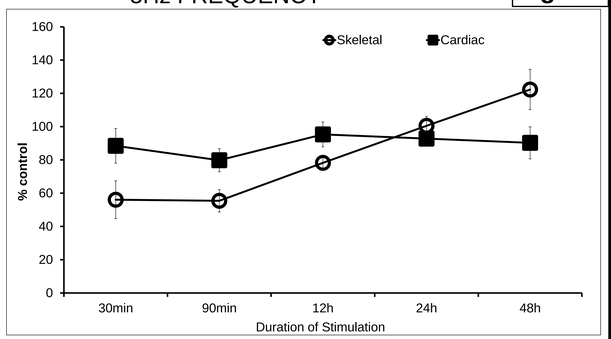
Electrical stimulation has opposite effect on intracellular GDNF in skeletal and cardiac muscle cells

Fig. 3A



A. Effect of 1Hz electrical stimulation (ES) on intracellular GDNF in skeletal muscle myotubes versus cardiac muscle cells. Similar to GDNF secretion, short-term ES had an opposing effects on the cell types.. The reduction of inhibitory effect or the activation of intracellular GDNF depended on the duration of stimulation. Values are presented as mean ± S.E.M (P ≤ 0.05).

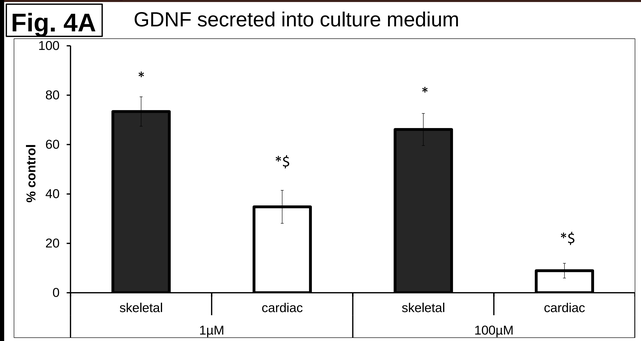
Fig.3B



B. Effect of 5Hz electrical stimulation (ES) on intracellular GDNF. 5Hz ES had only inhibitory effect on intracellular GDNF protein in cardiac muscle cells. In skeletal muscle cells, stimulation for 30min – 12h reduced GDNF levels although, this inhibitory effect started to be abolished when skeletal muscle cells were stimulated for 24h and 48h. Values are presented as mean ± S.E.M. (P≤ 0.05).

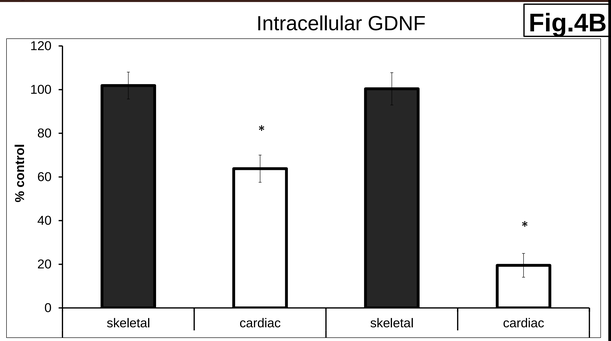
Acetylcholine has similar effects on GDNF secretion by skeletal and cardiac muscle cells

Fig. 4A



Effect of acetylcholine (ACh) on GDNF production by skeletal muscle and cardiac muscle. Culture medium containing 1µM or 100µM ACh was added to 5-day-old cardiac cells or 7-day-old myotubes. **Panel A) GDNF secreted into culture medium.** ACh inhibits GDNF secretion in both cell types although GDNF levels are more reduced in cardiac cells compared to skeletal muscle cells. GDNF protein concentration was determined by ELISA. Values are presented as Mean ± S.E.M. and Asterisk (*) indicates significance from control, dollar sign ($) indicates significance between the groups, (P ≤ 0.05).

Fig.4B



Panel B) Effect of ACh on intracellular GDNF in skeletal muscle and cardiac muscle. ACh decreased intracellular GDNF levels in cardiac cells but had no effect on GDNF content in skeletal myotubes. GDNF protein concentration was determined by ELISA. Values are presented as Mean ± S.E.M. and Asterisk (*) indicates significance from control, (P ≤ 0.05).

Electrical stimulation affects GDNF in skeletal muscle cells via voltage-gated sodium channels

Fig.5A

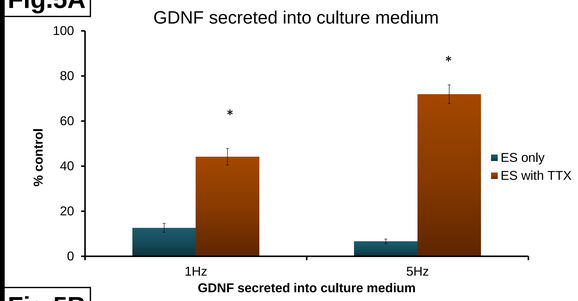


Fig.5B

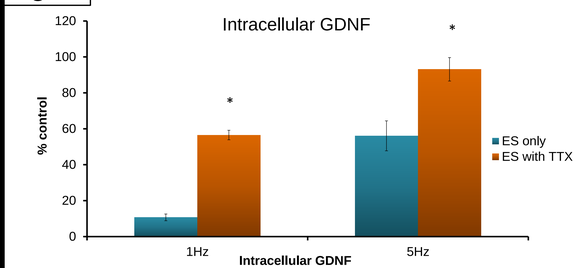


Figure 5. Blocking voltage-gated sodium channels in C2C12 moyubes. Cells were electrically stimulated with or without tetrodotoxin. The results showed that in cells stimulated in the presence of TTX, GDNF production was inhibited by 30% to 50%, whereas in cells stimulated without TTX, GDNF production was inhibited by 87% to 93%. **A.** GDNF secreted into culture medium and **B**, intracellular GDNF. GDNF concentration was determined by ELISA. Values are presented as mean ± S.E.M. (P≤ 0.05).

Summary and Conclusion

❖GDNF production in cardiac and skeletal muscle can be regulated by direct electrical stimulation and the effect appears to be dependent on activation of voltage-gated sodium channels in skeletal muscle cells.

❖The effect of electrical stimulation on GDNF production and release is dependent upon the frequency- and/or duration of stimulation in both skeletal and cardiac muscle.

❖ACh negatively regulates GDNF production in both muscle cell types. However, in skeletal muscle, ACh alters secretion but not intracellular content of GDNF. In cardiac muscle cells, treatment with ACh affects both the amount of GDNF secreted and that retained in cells.

❖The results suggest that GDNF production may be regulated differently in voluntary and involuntary muscles.

Reference

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Acknowledgements

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