Electrical stimulation has opposing effects on giall cell line derived neurotrophic factor expression in voluntary and involuntary muscles

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Abstract

GDNF cell line-derived neurotrophic factor (GDNF) is 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; Martinecci 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; Sarola 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.

Materials and Methods

1. Cell culture: Figure 1A & B show differentiated cardiac (C512) and skeletal myotubes (C2C12) in culture. Cells were grown and maintained in a water saturated incubator using 95% air and 5% CO2 during all electrical and chemical treatments. Cultures of conditioned medium and cells were collected between 0h and 24h 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 wells served as controls. Stimulation was at 3Hz or 5Hz with an approximate 24V 30ms pulse for durations between 30min and 4h. 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 electrolytic 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 preincubated with 1μM TTX and 10μM SR 95729 (TTX). Solutions 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

A. Effect of 1Hz electrical stimulation (ES) on GDNF secretion by skeletal muscle myotubes versus cardiac muscle myocytes. Cells were stimulated at 1Hz frequency. Short-term ES (30min-60min) inhibited GDNF secretion in myotubes while increasing GDNF in cardiac muscle cells. The inhibitory effect of ES 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).

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

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

A. Effect of acetylcholine (ACh) on GDNF production by skeletal muscle and cardiac muscle cells. Cultures were incubated with 1μM and 100μM ACh to determine if GDNF production was increased in skeletal muscle. Cells were stimulated with 10μM and 100μM ACh in the presence of conditioned culture medium and harvested cells were collected at 0h, 2h, and 24h.

B. Effect of ACh on GDNF production by skeletal muscle and cardiac muscle cells. Cells were preincubated with 1μM and 100μM ACh prior to electrical stimulation. Panel A) GDNF secreted into culture medium. ACh inhibits GDNF secretion in both cell types although GDNF production was 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. Panel B) GDNF protein secretion by skeletal muscle and cardiac muscle cells. ACh reduced GDNF production in both cell types although the inhibitory effect was more pronounced in skeletal muscle cells. GDNF protein content was determined by ELISA. Values are presented as mean ± S.E.M. and Asterisk (*) indicates significance from control. (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 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. 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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