Short Term Voluntary Exercise Alters GDNF Protein Expression in Rat Spinal Cord

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Short term voluntary exercise alters GDNF protein expression in rat spinal cord

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Abstract

Introduction

One major problem with aging individuals is the continuous loss of somatic motor neurons that innervate skeletal muscles. Increased amounts of physical activity have been shown to prevent the loss of motor neurons in aging individuals (Kanda and Hashizume, 1998).

GliaZ cell-line derived neurotrophic factor (GDNF) is one of the most potent trophic substances for motor neurons that innervate skeletal muscles. GDNF rescues somatic motor neurons from natural occurring cell death (Opperharm et al., 2000), rescues motor neurons from early neurodegenerative stages (Corse et al., 1995), and protects motor neurons from chronic degeneration (Corse et al., 1999). If GDNF protein expression is controlled by physical activity, then increasing levels of physical activity may reverse age-related changes of the motor neuron structure and function.

Hypothesis: Voluntary exercise will increase GDNF protein content of the spinal cord.

Methods

Aims

• Does GDNF protein content decrease in the spinal cord in aging animals?
• Does short term voluntary exercise increase GDNF protein content in the spinal cord?

Test Subjects

20 male Sprague-Dawley rats (Charles River Co.) aged 6 months (n = 12) and 2 years old (n = 11).

Sedentary (11 animals). Age-matched control animals had no running wheel access.

Exercised (12 animals): Animals were housed in individual cages with continuous access to voluntary running wheels for 2 weeks.

Determination of GDNF protein content:

Spinal cord samples were removed from rats 2 years of age. The lumbar region was processed. The sections were fixed in a buffer that was homogenized in a buffer salt solution containing protease inhibitors followed by centrifugation at 23,700 g for 30 minutes. The supernatant was collected and GDNF protein content was measured using an enzyme-linked immunosorbent assay (ELISA). GDNF values were quantified using a known standard curve and expressed as pg of total.

Statistical analysis:

Data are represented as mean ± standard error of the mean (SEM). Data were analyzed using one-way ANOVA to test for differences among the independent groups. Significance was established as p < 0.05.

Immunohistochemistry

Spinal cord sections were removed and fixed in 4% paraformaldehyde overnight at 4°C. Tissues were then washed in phosphate buffered saline (PBS) and placed in a 30% sucrose solution overnight at 4°C for cryoprotection. Tissues were stored at -80°C until ready for processing. The spinal cord was sectioned on the cryostat. Fixed tissues were incubated with antibodies against GDNF, vacuolar protein sorting (VPS) 20, and calretinin to visualize neuronal cell bodies and GABAergic, cholinergic, and GABAergic motor neurons, respectively. Sections were placed on slides and viewed using a confocal microscope.

Western Blot

Homogenized tissue supernatant was used to determine protein concentration using a BCA protein assay with bovine serum albumin as the protein standard. Samples were prepared for loading into polyacrylamide gels by adding Laemmli 2X Loading buffer and boiled for 5 minutes. A protein ladder and a positive control of a GDNF standard were added to the gel. The gel was run at two different voltages from a power source. 100 Volts through the stacking gel and 200 Volts through the loading gel. Once the gel was run, the gel was transferred to a polyvinylidene difluoride (PVDF) membrane where it was transferred to a power supply of 12 Volts for 1 hour. Once the transfer finished, bands from the protein ladder were marked for visualization, and the PVDF membrane was blocked non-specifically for 1 hour at 4°C under agitation. The membrane was incubated with a primary antibody against GDNF overnight at 4°C under agitation. The membrane was washed under agitation, then incubated with a HRP-conjugated secondary antibody for 1 hour at 37°C under agitation. The ECL detection kit was used to visualize the proteins and X-ray films were developed.

Results

Figure 1: Immunohistochemical staining of the spinal cord for GDNF in 2 year old control and exercised animals (6 months). Scale bar = 50 μm.

Figure 2: GDNF protein content in spinal cord measured via ELISA. Spinal cord was taken from both control animals aged 1 year and exercised animals with 6 weeks of voluntary exercise. Data represent the mean SEM (p < 0.05).

Figure 3: GDNF protein content in spinal cord measured via ELISA. Spinal cord was taken from both control animals aged 2 years and exercised animals with 2 weeks of voluntary exercise. Data represent the mean SEM (p < 0.05).

Figure 4: Lumbar segments of the spinal cord from 2 year-old control animals were fixed and bound with rabbit anti-GDNF (green), mouse anti-CHAT (red), and goat anti-CGRP (blue) and were viewed on a confocal microscope.

Figure 5: Lumbar segments of the spinal cord from 2 year-old animals that exercised for 2 weeks. Sections were fixed and bound with rabbit anti-GDNF (green), mouse anti-CHAT (red), and goat anti-CGRP (blue) and were viewed on a confocal microscope.

Figure 6: Western blot using antibodies specific for GDNF. Spinal cord was taken from control animals aged 6 months and 6 month old animals with 2 weeks of exercise. Lane 1 is the 6 month old control animal. Lane 2 is the 6 month old control animal.

Discussion

Increased voluntary running-wheel activity in adult and aged rats increases GDNF protein content in the spinal cord.

Conclusions

These results show that short term voluntary exercise increases GDNF protein levels in the spinal cord of young and old animals. Immunohistochemical results suggest that motor neuron size is affected by exercise. Our western blot results suggest that expression of GDNF is higher following voluntary exercise in the spinal cord.

Short term voluntary exercise may increase GDNF protein content in the spinal cord of aged individuals and may act as a measure to prevent motor neuron loss commonly associated with senescence.

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