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## Effects of Nerve Growth Factor on Acetylcholinesterase Activity of Injured Spinal Cord

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**Abstract:** Fourteen rats were examined and divided into two groups of 7: the control (Group a) and NGF-treated animals (Group B). All animals received a 50 g clip-compression injury to the spinal cord at the T9 level. In the NGF-treated animals, NGF (0.1 mg/kg NGF) was injected subcutaneously for seven days. After spinal cord injury, the mean AChE activity was

$41.47 \pm 1.7122$  activated substrate min/ml/gr. wet tissue and  $47.52 \pm 1.471$  in NGF-treated animals. The difference between Groups A and B was statistically significant ( $p < 0.01$ ). These results suggest the possible role of NGF in the cholinergic system.

**Key Words:** Acetylcholinesterase, Nerve Growth Factor, Spinal Cord Injury.

### Introduction

The ongoing search for neurotrophic factors for motoneurone survival and function. Nerve growth factor (NGF) exerts neurotrophic activity after binding to the NGF receptor and being internalised and retrogradely transported to the neuronal cell body as NGF/NGF receptor complex (2). In some studies, it has been demonstrated that NGF has an effect on acetylcholinesterase (AChE) enzyme in both culture mediums (6, 9, 13, 15, 16) and animal experiments (6, 11, 14, 16, 19). Human NGF has prevented the degeneration of basal forebrain cholinergic neurones in primates (15, 17).

In the present study the spinal cord was injured in order to observe the effects of a single dose of NGF on the content of the AChE activities of these tissues on day one and seven.

### Materials and Methods

Fourteen winstar rats, weighing 230-300 g, were divided into two groups of 7: the control (Group A) and NGF-treated animals (Group B).

The animals were anaesthetised with ketamin hydrochloride (75mg/kg, intraperitoneally). Under aseptic conditions, a T8-10 laminectomy was performed and the each rat received a 50 gr clip (Aesculap, Germany, Yaşargil Clips) compression injury to the cord with microsurgery at approximately the T9 level. A single dose of NGF (SIGMA Chemical Company, USA) (0.1 mg/kg)

was injected in each animal in the NGF-treated group subcutaneously for seven days. In the control animals, equal volumes of serum physiologic were injected subcutaneously.

Spinal cord tissues were obtained from the groups on the 7<sup>th</sup> day, 12 hours after the last treatment with placebo or NGF. To determine the levels of AChE, the spinal cord were rapidly removed. They were approximately 2 cm long. All samples were stored at -70°C until they were assayed.

#### AChE activity measurements

The tissues were homogenised in a Potter-Elvehjem homogenizer (20mg tissue for 1 ml, pH 8, 0.1 M phosphate buffer). 0.4 ml of this homogenate was placed in a spectrophotometer cuvet containing 2.6 ml (pH, 0.1 M) phosphate buffer. 100µl DTNB (5,5-dithiois (2-nitrobenzoic acid), Elmon's Reagent) was added. Absorbency was read at 412 nm. Thus absorbency was set to zero.

2 µl substrate was added and the reaction begun. Changes in absorbency were recorded every minute for at least 6 minutes. Enzyme was calculated using following formula.  $R = 5.74 \times 10^{-4} \times (A/Co)$  (where R is the rate, A is changes of absorbance per minute and Co is the concentration of the tissue (mg/ml) (3).

#### Statistical Analysis

AChE activity was expressed as the means and standard deviation of the means of the the results of the

measurements. Statistical analysis of the comparisons between groups was performed using the one-way unpaired t test.

**Results**

*AChE Activity in Contusive Spinal Cords*

After spinal cord injury, the mean AChE activity was  $41.74 \pm 1.722$  activated substrate min/ml/gr. wet tissue in the controls and  $47.52 \pm 1.471$  in the NGF-treated animals (Table 1, Fig 1). The difference between two groups was statistically significant ( $p < 0.01$ ).

Table 1. Mean AChE Activity in Spinal Cord Injury (Activated substrate min/ml/gr wet tissue)\*

	N. of animals	7th Day
Control Animals	7	$41.13 \pm 2.99$
NGF-treated Animals	7	$47.7 \pm 5.75$

\*= Statistically significant ( $p < 0.001$ ).

**Discussion**

It is a well-known fact that cholinergic neurones are widely distributed in the spinal cord (1, 4, 12). It is also well established that both preganglionic autonomic neurone and somatic motoneurones are cholinergic (1, 4, 10) and make up a significant proportion of the total neuronal pool (1). Acetylcholinesterase, the enzyme that catalyzes a reaction of hydrolysis of acetylcholine to choline and acetate (7), is regarded as specific marker for cholinergic function (7, 8, 18) and is used as a differentiation marker (19). Bakhit et al. reported that NGF has a role in the spinal motoneurones. They also determined NGF receptors in rat spinal motoneurones during development and observed that contusion injury of the spinal cord resulted in increased expression of the NGF receptor mRNA (1). Brunello et al. also reported that this NGF receptor mRNA increases in the contused rat spinal cord (2). Fernandez et al. infused NGF locally on the

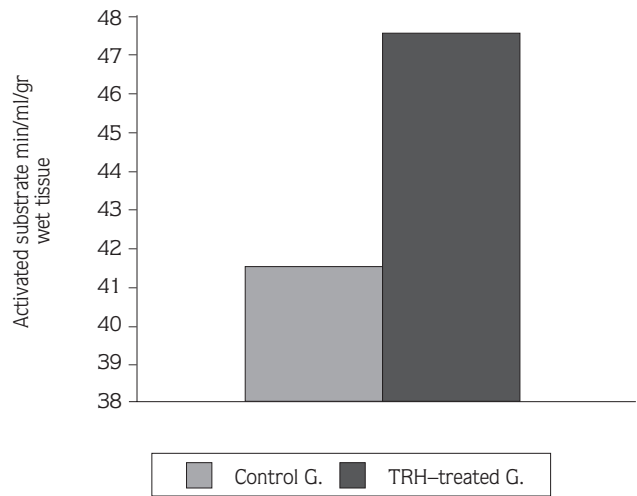


Figure 1. Graph showing AChE Activity levels in both groups. This difference is statistically significant ( $P < 0.01$ ).

transected spinal cord in adult rats and observed increased induction of axonal sprouting by histological staining in NGF-treated animals (5).

In one study, it was demonstrated that NGF increases AChE activity and this activity increment reflects the extent of the fiber network of the cholinergic neurones (9). Thus, the extent of the spinal cord injury may correlate with the extent of reduced function of the cholinergic neurones and, hence, in AChE activity.

Our findings show that AChE levels in injured spinal cords are more significantly suppressed the 7<sup>th</sup> day in the control animals than in the NGF-treated group. This supports the results of the studies cited above and is in accordance with them. In the NGF-treated animals, some decrease in AChE activity was observed on the 7<sup>th</sup> day. The difference in the AChE activities of the control and the NGF-enhancing effects on AChE activity and the possibility of a role for NGF in promoting the recovery of function after spinal cord injury.

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