# Effect of Dipeptide N-Acetylaspartylglutamate on Denervation-Induced Changes in the Volume of Rat Skeletal Muscle Fibers

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N-acetylaspartylglutamate prevents the denervation-induced increase in the volume of muscle fibers in rat diaphragm, the phenomenon being more pronounced for the hydrolysable isomer. The effect of dipeptide manifested against the background of blockade of metabotropic glutamate receptors. It was hypothesized that N-acetylaspartylglutamate is involved in the regulation of the volume of skeletal muscle fibers via activation of ionotropic receptors by both dipeptide and glutamate molecules.

**Key Words:** *N-acetylaspartylglutamate; skeletal muscle; muscle fiber volume; glutamate receptors*

Motoneurons regulate activity of some ion-transporting systems in the sarcolemma. One of these systems is furosemide-sensitive $Na^+$-, $K^+$-, $Cl^-$-co-transport not only involved in generation of the negative charge on the muscle fiber (MF) membrane, but also affecting water homeostasis in cells [1]. Activation of $Na^+$-, $K^+$-, $Cl^-$-cotransport is one of the key stage in the development of denervation-induced alterations in MF, including the increase in MF volume [1,6]. It was previously found that the intensity of this co-transport is controlled by the inhibitory action of the motoneuron realized via activation of glutamate receptors located on the sarcolemma [2,6].

Motor nerve terminals in rats contain dipeptide N-acetylaspartylglutamate (NAAG) [3] participating in the synaptic transmission as an agonist of ionotropic glutamate NMDA- and metabotropic mGlu3-receptors [7,8] or as a glutamate precursor produced during hydrolysis catalyzed by glutamate carboxypeptidase II [4,5].

Our aim was to study the possible role of NAAG in the neurotrophic control of MF volume.

## MATERIALS AND METHODS

Experiments were carried out under ether anesthesia on random-bred male albino rats weighing 160-180 g. The phrenic muscle was isolated and cut into fragments placed in Petri dishes with 199 medium. pH was maintained at 7.2-7.4 with HEPES (Sigma).

The following reagents were added to the medium: α-NAAG (hydrolysable isomer, 10 μM, Sigma), β-NAAG (non-hydrolysable isomer, 10 μM, Sigma), and (2S)-α-ethylglutamic acid (EGLU, 100 μM, Sigma), an antagonist of metabotropic mGlu2- and mGlu3 glutamate receptors. The dishes were incubated at 37°C in a humid atmosphere consisting of 95% $O_2$ and 5% $CO_2$ for 3 h, thereafter the muscle fragments were placed into liquid nitrogen. Ultrathin cross-sections (7 μ) were cut in the cryostat. After dehydration, the sections were stained with hematoxylin and eosin (Sigma) and photo-

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graphed with a camera mounted on a Leica DMLS/MPS-32 light microscope. The photos were used to calculate the area of MF cross-section and to assess changes in the volume of MF.

The results were analyzed statistically using Student's *t* test at $p<0.05$.

## RESULTS

Nerve transection and 3-h incubation of the muscle fragments significantly increased MF cross-section (Fig. 1). Hydrolysable α-NAAG in the medium completely prevented the increase in MF volume caused by denervation (Fig. 1). The non-hydrolysable isomer β-NAAG in the same concentration produced less pronounced effect. These data suggest that the effect of the neuropeptide is mediated by products of its hydrolysis (glutamate and/or N-acetylaspartate). We previously demonstrated that glutamate participates in the regulation of MF volume via activation of NMDA receptors [2]. However, the effects of NAAG can be mediated by activation of not only NMDA-, but also mGlu3-receptors. In the incubation medium, EGLU had no effect on denervation-induced increase in MF volume (Fig. 1) in contrast to α-NAAG applied against the background action of this antagonist ($p<0.05$). Thus, the metabotropic mGlu3-receptors are not involved in the realization of NAAG effect on the postdenervation changes in the volume of skeletal MF.

These data suggest that NAAG neuropeptide participates in the regulation of skeletal MF volume. The mechanism of neurotrophic effect of NAAG can be realized via activation of NMDA-receptors (but not mGlu-receptors) by this dipeptide and glutamate formed during its hydrolysis by glutamate carboxypeptidase II [5]. Activation of postsynaptic NMDA-receptors stimulates the synthesis of NO in sarcoplasm, which inhibits $Na^+$-, $K^+$-, $Cl^-$-cotransport in MF membrane [2,6]. Disturbances in this mechanism lead to an increase in MF volume due to enhanced entry of $Cl^-$ ions.

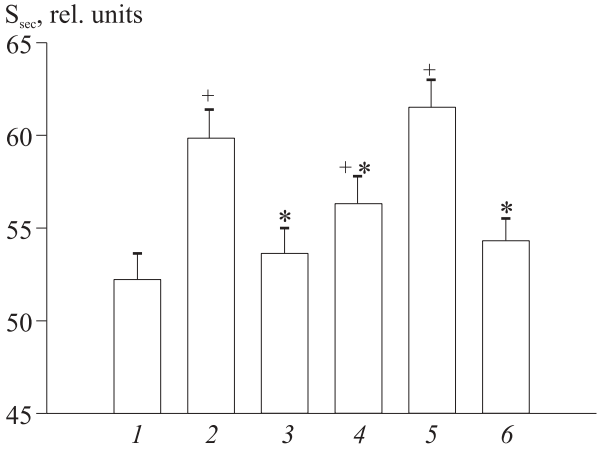


**Fig. 1**. Cross-section area ($S_{sec}$) of innervated MF (*1*) and that of MF incubated for 3 h in 199 medium (*2*) or in this medium with α-NAAG (*3*), β-NAAG (*4*), EGLU (*5*), and EGLU+α-NAAG (*6*). $p<0.05$ compared to $^+$(*1*) and *(*2*).

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