Effect of Tetrapeptide Cortagen on Regeneration of Sciatic Nerve

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Intramuscular injection of 10 μg/kg cortagen to rats during 10 days after transsection and suturing of the sciatic nerve increased the growth rate and conduction velocity in the regenerating nerve fibers by 27% and 40%, respectively.

Key Words: *peptides; cortagen; regeneration; nerve*

Regeneration of peripheral nerves after traumatic disruption of the nerve trunk is a slow process, and in many cases it does not culminate in complete functional recovery of the damaged nerve. Thus, new methods and tools stimulating regeneration of the nervous tissue are an actual problem.

Since the discovery of the nerve growth factor [9], various peptides and oligopeptides were identified, which promote the neurite growth *in vitro* [10], stimulate regeneration of the peripheral nerves, and activate reinnervation of the target tissues *in vivo* [3,7, 8,11-13].

The electrophysiological, morphological, biochemical, and functional tests revealed positive effects of the derivatives of polypeptide precursor proopiomelanocortin: melanocyte-stimulating hormone-α, ACTH-like peptides, and opioid peptides (Leu-enkephalin and its synthetic analog dalargin) on various parameters of regenerating nerve [1]. The effect on various parameters depended on structure, physicochemical properties, dose, and mode of application of the peptide.

A polypeptide cortexin isolated from the cerebral cortex [5] produces a neurostimulating effect in organotypic culture of chicken embryo brain [5]. It also stimulates reparative processes in the brain of neurological patients and promotes recovery of brain activity after stress, which attests to its neurotrophic activity.

Our aim was to study the effect of a new synthetic tetrapeptide cortagen on functional recovery of damaged sciatic nerve (SN).

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=25) weighing 200-250 g. Surgery was performed under sterile conditions and Ketamine anesthesia (45-50 mg/kg Ketamine and 10-20 mg/kg Rometar intramuscularly). SN trunk was transected proximally to its division into three major branches (tibialis, peroneus, and suralis), at the site of separation of the cutaneous branch from the common trunk. The nerve was sutured under a MBS-2 microscope with three epiperineural microsurgical sutures using an atraumatic needle and the 10/0 supramide thread.

Cortagen (10 μg/kg, intramuscularly) was injected daily during 10 days starting from the day of surgery. The control rats were injected with physiological solution according to the same protocol.

Cortagen (Ala-Glu-Asp-Pro) was obtained by directed synthesis based on amino acid analysis of cortexin.

The effect of cortagen on recovery of nerve conduction in damaged SN was assessed by compound action potential (CAP) 4 weeks after surgery. The test

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was carried out *in vitro* by the method of Legrane on a special ebonite multielectrode platform [2]. The method made it possible to calculate CAP parameters under standard conditions and to locate the site of regeneration by recording CAP at a distance of up to 30 mm from the stimulating electrodes. The dissected nerve was placed on the platform so that the suture strictly coincided with the reference electrode. Then the platform was filled with mineral oil warmed to the body temperature. The proximal end of SN was stimulated with rectangular pulses (0.2 msec duration) generated by an ESU-2 electrical generator. The amplitude of stimuli was 3-fold threshold value. CAP was recorded distally from the suture. The signal was preliminary amplified and fed into an C1-69 oscilloscope for monitoring and recording.

The length of the nerve fragment with restored conduction and the latency of CAP were measured and the conduction velocity in the regenerating nerve was calculated. The results were analyzed statistically using Student's *t* test.

RESULTS

Electrical stimulation of intact SN induced CAP spreading along the entire nerve fragment.

The electrical testing of regenerating nerve was performed 4 weeks after surgery. This period is optimal to reveal the effects of various factors on recovery of conductance in regenerating nerve [3]. At this time, the distance, at which the nerve conductance was restored, can be precisely determined. This distance corresponded to the length of the regenerating nerve fibers. It should be noted that this value is not absolute, because some fine regenerating fibers can grow for a larger distance, but their activity can be shunted in the trunk, and they not contribute into CAP.

The animals not injected with cortagen demonstrated pronounced variations in the growth rate of nerve fibers, which agrees with the fact that the intensity of regeneration processes depends on many factors. Some factors (sex, age, site and degree of damage) can be specified in the experiment, while others (hormonal and immunological status) are responsible for pronounced individual variations in the rate of reparative processes. However, in cortagen-treated rats the growth rate of nerve fibers varied only negligibly and corresponded to the upper limit of the fiber growth rate in the control rats (Table 1). Supposedly, cortagen has a positive effect on the regeneration processes in animals with low fiber growth rate. A comparison of the mean values of the growth rate of regenerating nerve fibers revealed a significant increase of the nerve length with the restored conduction (by 27%, $p<0.01$, Table 1).

TABLE 1. Effect of Cortagen on Growth of Regenerating Fibers in Damaged Sciatic Nerve in Rats

Index		Control	Cortagen
Restoration of nerve conduction, mm	*M*±*m*	18.3±1.5	23.3±0.4*
	range	14-24	22-24
Conduction velocity, m/sec		13.9±1.6	19.4±1.1**

Note. *$p<0.01$ and **$p<0.02$ compared to the control.

To assess the latency and conduction velocity, we used potentials recorded at a distance of 12 mm from the reference electrode. At this distance all rats demonstrated stable CAP. The regenerating fibers in damaged SN in the control and experimental groups had pronouncedly lower conduction velocity (by 66 and 53%, respectively) than in intact rats. Low conduction velocity observed at the initial stage of regeneration results from poor myelination and small diameter of the growing fibers. However, the conduction velocity in the experimental group was significantly higher than in the control group ($p<0.02$, Table 1). These data attest to a stimulating effect of cortagen on regeneration in damaged SN.

Cortagen also promoted functional maturation of nerve fibers, which manifested in a 40% increase of conduction velocity. SN consists of at least three groups of nerve fibers (A, B, and C), different in their function and conduction velocity [4]. Thick myelinated A-fibers regenerate more rapidly than thin non-myelinated C-fibers. Among the population of A-fibers, the motor fibers regenerate faster than the sensory fibers [6]. Therefore, the major contribution into CAP in our experiments was probably made by sensory and motor A-fibers.

Therefore, our findings indicate that tetrapeptide cortagen possesses a pronounced neurotrophic activity. Intramuscular injection of cortagen significantly promotes structural and functional recovery of the damaged peripheral nerve.

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