The Effects of Local Fetal Brain Extract Administration on the Electromyogram of Crushed Sciatic Nerve in Rat

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ABSTRACT

To evaluate the effects of local administration of the fetal brain extract (FBE) on the crushed peripheral nerve, 15 male Wistar rats were used. The right sciatic nerve of 10 rats was locally underwent the tense compression for 30 seconds and then randomly divided into experimental and control (sham) groups. The remained rats were considered as intact animals. Treatment was commenced weekly, by intramuscular injection of FBE around the injured site of experimental rats, started from the 1st week post-operation to the 5th week. The control (sham) rats were injected normal saline in the same manner. The delay time of the electromyographical records in different groups was measured from stimulation-response curves. This delay time was obtained by stimulating of the distal part of the sciatic nerve and recording the electrical response in the plantar muscles, using a computerized oscilloscope. The results indicating that the administration of FBE may improve dramatically the regeneration rate of the sciatic nerve in the early stage of the recovery period. *Iran. Biomed. J. 5 (2 & 3): 73-77, 2001*

INTRODUCTION

While considerable emphasis has been placed on the importance of the technical aspects of the surgical nerve repair, it has been suggested that the rate of neuronal regeneration is influenced by neurotrophic factors such as Nerve Growth Factor (NGF) [1, 2], Brain Derived Neurotrophic Factor (BDNF) and Neurotrophin3 (NT3) [3, 4]. This neurotrophic hypothesis was originally in 1898 [5]. A series of recent studies have supported this hypothesis and the concept that with a crush, the peripheral nerve under the influence of neurobiological factors is capable of regeneration with significant specificity [6]. In this subject, recent experimental studies in adult rodents indicate that neurons of many regions of the brain and spinal cord are capable of extensive axonal growth when implanted into the fetal Central Nervous System (CNS) [7, 8]. Also, it has been shown that survival of motoneurons, particularly in immature animals, depends on peripheral connections providing trophic support [9]. After peripheral axotomy, some motoneurons in rodents survive and undergo typical reactive changes classic for chromatolysis, while others undergo changes that lead to cell death [10]. This motoneuronal death is characteristic of apoptosis, similar to that of neurons after trophic factor withdrawal [11]. The discovery and characterization of NGF led to the neurotrophic theory [12]. To support this theory, it has been demonstrated that neuronal death after sciatic nerve transection could be prevented in postnatal rats with systemic administration of nerve growth factor [13] and in adult rats with locally supplied NGF at the site of nerve section [14]. Multiple growth factors have shown similar effects on neuronal protection of facial motoneurons [15]. Similar observations have been obtained by application of BDNF and NT3, which indicate the improvement of motoneuronal survival from 14% to 49% and from 14% to 29%, respectively, 1 week after facial axotomy in newborn rats [16]. The most dramatic protectional effects of neurotrophics are reported by local application of glial-cell-line-derived factor and growth factor replacement that provides 100% protection of facial motoneurons after axotomy [17].

Although transplantation of adult neurons into fetal CNS has exhibited putative effects of fetal CNS for extensive axonal growth [7, 8], the current research examines, electromyographically, the

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effects of fetal brain extract (FBE) on the regeneration of prepheral nervous system (PNS).

MATERIALS AND METHODS

Animal model and experimental design. Fifteen adult male Wistar rats from our stock were maintained under conditions of controlled lighting (lights on: 6 a.m. to 6 p.m.) with free access to standard laboratory food (JavanehKhorassan, Iran) and tap water.

Five rats were kept as intact animals and 10 underwent sciatic nerve compression and then randomly assigned as control and experimental animals (n = 5 in each group). Each animal was coded by ear marked. All assessments were made in a blinded fashion and decoded at the end of study.

Surgical procedure. The animals of experimental and control groups were anesthetized with intraperitoneal injection of 0.2 ml of a mixture (1:2) of 10% ketamine (Bayer, Germany) and 2% xylazine (Boxtel, Holland). For axotomy, the right sciatic nerve (SN) was exposed through a gluteal muscle splitting incision. At this location, the nerve trunk was crushed for 30 seconds between prongs of #5 clamp forceps. The muscle and skin were then closed with 14-mm stainless steel sutures.

Preparation of FBE. Twenty virgin Wistar rats were mated. On the 17th day of pregnancy, the pregnant rats were sacrificed and their fetuses were removed immediately from uterine horns. The whole brain of each fetus was removed from the skull and homogenized. The volume of homo-genized brain was reached to 1 ml by adding saline, and then centrifuged for 10 min. The extract was pulled and stored in Eppendorf tubes at -20°C.

FBE application. The weekly injections of FBE were applied around the injured site of sciatic nerve of experimental rats. Injection was started at 7th day and ended at 35th day post-operation. At the time of injection, the Eppendorf tubes containing FBE were warmed to room temperature. Then, 0.2 ml of FBE was injected intramuscularly around the injured site. Control rats received an equal volume of normal saline in the same manner.

Electromyographical assessment. To evaluate the effects of FBE on the recovery of crushed sciatic nerve fibers, the delay time of compound muscular action potentials (CMAP), during a period of 72 days, was measured in experimental, control and intact rats, as an index of regeneration.

The electromyographical assessment of regeneration, using a comprehensive electro-physiological teaching unit (CEPTU) oscilloscope-linked computer, was started from the 5th week post-operation (no record was available during the earlier weeks). To measure the regeneration rate in distal part of sciatic nerve, the CMAP were recorded from plantar muscles, via a bipolar recording needle electrode which was inserted into the plantar muscles. To evoke action potentials, the sciatic nerve was precutaneously stimulated by a bipolar plotted stimulating electrode, with a square pulse of 0.1 ms duration and 20 volt in magnitude. The stimulating electrode was adjusted until a saturated response was recorded. At this location, the recorded CMAP were saved. Then, the recorded CMAP from animals, in each group and at each stage, were averaged and a printout of each was taken. Finally, the delay time (latency) of averaged records of experimental and control rats were measured and compared with each other and with intact animals.

The latency was determined by measuring the distance between electrical pulse artifacts and the onset of CMAP.

Statistical analyses. Results are expressed as mean ±SEM. Statistical analyses were performed using one way ANOVA with Student's *t*-test in order to assess the significance of differences between individual means.

RESULTS AND DISCUSSION

The averaged delay time of CMAP of control and experimental animals, during experimental period (72 days) are presented in Table 1. In Figure 1, the averaged CMAP of intact (a), experimental (b) and control (c) animals are plotted in different stages (35, 49 and 72 days post-operation). The averaged delay times show a shorter latency in records of experimental animals than controls during the experimental period (Table 1).

However, by passing time, the latency of control group became closer to experimental group. Data presented in Table 1 also shows that the delay time of final (72 days post-operation) averaged record of

74

Table 1. The comparison of averaged delay time (latency, msec) during the experimental period in control and experimental animals.

Record no.	Recording time (day•)	Control group	Experimental group
1	28	No record available	No record available
2	35	5.15 (± 0.13)	2.86 (± 0.08) ***
3	42	4.55 (± 0.09)	2.64 (± 0.08) ***
4	49	3.60 (± 0.11)	2.40 (± 0.05) **
5	56	3.35 (± 0.1)	2.37 (± 0.04) **
6	72	3.50 (± 0.08)	2.34 (± 0.08) **

$**P<0.01$, $***P<0.001$ compare with the control group (Student's *t*-test) . The averaged delay time in intact animals was measured 1.78 (± 0.1) msec. The values are presented as Mean + SEM. •post-operation days
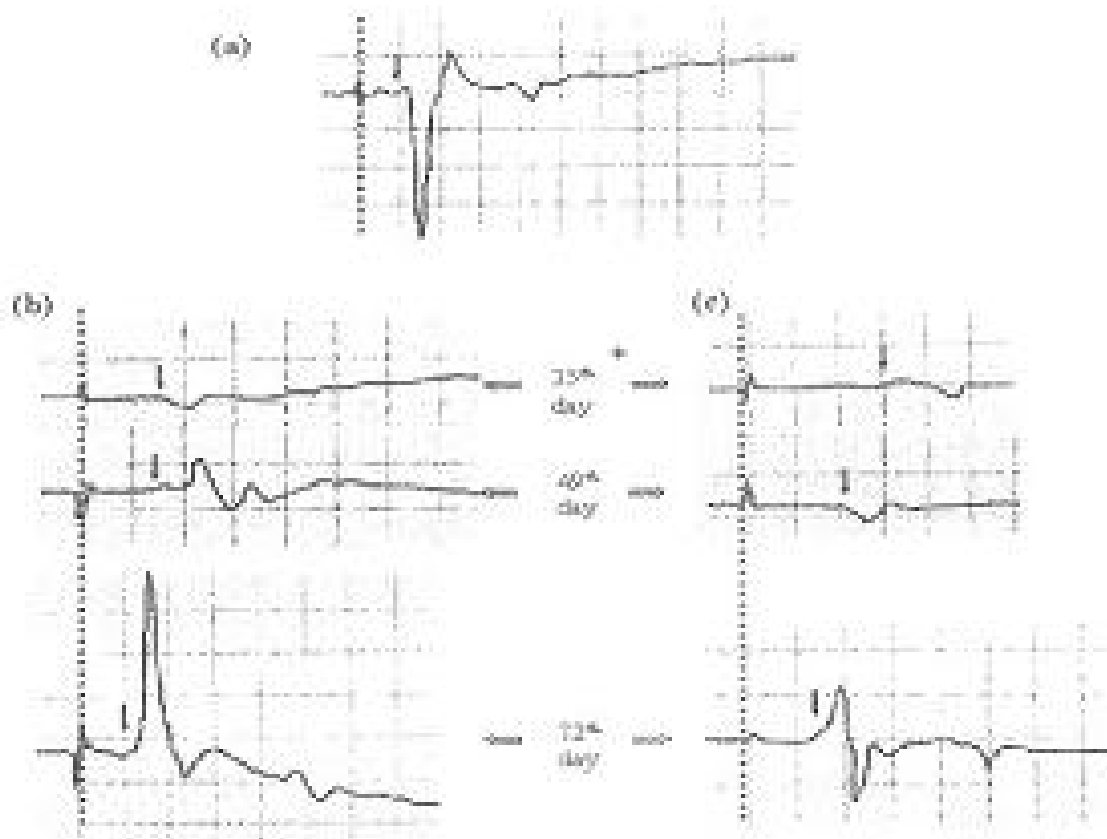

Fig. 1. (a) An averaged CAMP recorded from an intact animal. Averaged CAMP at different stages (days) of experimental period, recorded from experimental (b) and control animals (c). The time scale is 2 msec. The stippled lines show the stimulus time. *The days are post-operation. ↓The onset of CAMP.

75

experimental group (2.35 msec) is very close to the intact animals (1.8 msec) than control group (3.5 msec). Although the principal mechanisms underlying cell death following peripheral nerve injury are currently unknown, a major contributing factor is likely to be deprivation of target-derived neurotrophic factors such as the neurotrophins [18]. Neuronal death after sciatic nerve transection can be prevented in postnatal rats with systemic administration of NGF [13] and in adult rats with locally supplied NGF at the site of nerve section [14]. There have been numerous reports that neurotrophins and NGF rescue motoneurons subjected to transection or crush of peripheral nerves [2, 15-17, 19]. Also, it has been shown that motoneurons respond to neurotrophins*in vitro* [18, 20] as well as retrogradely transport neurotrophins [21, 22]. It seems that the occurrence of neuronal loss following axotomy in an age-related fashion, parallels also the age-related loss of trophic factors [23].

The goal of this research was to examine the effects of FBE on recuperation of motor function in the early phase of regeneration. On the base of electromyographical records, the delay time (conduction velocity) was measured on different post-operation days (Table 1). This parameter was used to evaluate the recovery of injured nerve which may include the regeneration of nerve fibers and formation of the new myelin sheet. The results obtained show that during the early phase of the recovery, the application of FBE has a beneficial effect on the functional recovery and appearance of electrical activity of plantar muscles; the shorter the delay time the higher the conduction velocity. As are shown in Figure 1 and Table 1 the latencies, especially in earlier stages, are shorter in the experimental than in control group.

By appearing of different peaks in CMAP of experimental group (stage 49 days) it could be concluded that FBE application may enhance the regeneration of nerve fibers with different diameters and/or myelin sheet formation.

These results demonstrate that application of FBE may have dual effects; firstly, with retrograde transportation of FBE, the neuronal cell bodies rescue of cell death and therefore could regenerate the damaged fibers (neurotrophic effect) [7, 8] and secondly, with regional and anterograde effects of FBE, the Schwann cells may keep their activities and start to re-make myelin sheet (biostimulator effect) [8]. It has been shown that local application of brain-derived neurothrophin factors prevents the death of motoneurons in newborn rats [16]. These results suggest that the beneficial effects of FBE is more evident in the early phase of peripheral nerve recovery; the period which is critical for re-establishment of end organ innervation.

In conclusion it may be concluded, similar to the effects of fetal brain transplantation on the adult CNS regeneration, FBE may also enhance the PNS regenration.

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