Jpn J Ophthalmol 2009;53:257–266
DOI 10.1007/s10384-009-0657-8

LABORATORY INVESTIGATION

Axonal Regeneration Induced by Repetitive Electrical Stimulation of Crushed Optic Nerve in Adult Rats

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Abstract

Purpose: To investigate whether electrical stimulation promoted axonal regeneration of retinal ganglion cells (RGCs) after optic nerve (ON) crush in adult rats.

Methods: Transcorneal electrical stimulation (TES), which stimulates the retina with current from a corneal contact lens electrode, was used to stimulate the eye. TES was applied for 1 h immediately after ON crush. Axonal regeneration was determined by anterograde labeling of RGC axons. To examine whether the axonal regeneration was mediated by insulin-like growth factor 1 (IGF-1) receptors, an IGF-1 receptor antagonist, JB3, was injected intraperitoneally before each TES application. Immunostaining for IGF-1 was performed to examine the effects of TES. To test the survival-promoting effects of TES applied daily, the mean density of retrogradely labeled RGCs was determined on day 12 after ON crush.

Results: Compared with sham stimulation, the mean number of regenerating axons significantly increased at 250 μm distal from the lesion and increased IGF-1 immunoreactivity was observed in retinas treated daily with TES. Preinjection of an IGF-1 receptor antagonist significantly blocked axonal regeneration by TES applied daily. TES applied daily also markedly enhanced the survival of RGCs 12 days after ON crush.

Conclusion: TES applied daily promotes both axonal regeneration and survival of RGCs after ON crush. **Jpn J Ophthalmol** 2009;53:257–266 © Japanese Ophthalmological Society 2009

Key Words: axonal regeneration, insulin-like growth factor 1, optic nerve crush, retinal ganglion cells, transcorneal electrical stimulation

Introduction

Electrical stimulation (ES) has been used to control neuronal activities, which play a critical part in the development and plasticity of neural circuitry.[1,2] Some, but not many, reports have indicated that ES has regenerative effects on damaged neural tissues. For example, brief ES applied to a crushed femoral nerve accelerates the speed of axonal regeneration in adult rats.[3] ES also promotes axonal regeneration and recovery of neurological functions after spinal cord injury.[4,5] Although retinal ganglion cells (RGCs) have been an experimental model for regeneration of central nervous system (CNS) axons, only one in vitro experiment has demonstrated that growth of RGC axons is enhanced by ES combined with brain–derived neurotrophic factor, [6] and the question as to whether ES can promote regeneration of the optic nerve (ON) in vivo remains to be answered.

Received: September 9, 2008 / Accepted: January 30, 2009
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Previous studies have shown that survival of RGCs after ON transection is promoted by brief ES applied to the stump,[7,8] and additional work has shown that transcorneal ES (TES), which stimulates the retina via the cornea, promotes the survival of both axotomized RGCs and degenerated photoreceptors in the rat retina, and that the underlying mechanism of the survival-promoting effect of TES on axotomized RGCs is the elevation of retinal insulin-like growth factor 1 (IGF-1), possibly secreted by Müller cells.[9,10]

IGF-1 is known to promote neurite outgrowth and regeneration. In the peripheral nervous system (PNS), IGF-1 is present in both the neurons and the skeletal muscles, and acts as a neurotrophic factor for motor, sensory, and sympathetic neurons to promote growth cone motility and neurite outgrowth.[11–13] It is also known that IGF-1 promotes neurite outgrowth in explants of adult rat retinas.[14] Such available evidence makes it clear that IGF-1 plays an important role in axonal regeneration and survival of CNS neurons as well as of those in the PNS. It is possible to stimulate the retina repetitively with ease when TES is performed with a corneal electrode, and repetitive TES may enhance and prolong the upregulation of retinal IGF-1 levels, thus promoting axonal regeneration of RGCs.

In the present study, we tested the hypothesis that TES can promote axonal regeneration after ON crush in adult rats through activation of the IGF-1 pathway. The results showed that daily application of TES promoted both axonal regeneration and survival of axotomized RGCs. TES applied daily upregulated retinal IGF-1 levels, and at the same time, axonal regeneration was completely blocked by an IGF-1 receptor antagonist, whereas the promotion of RGC survival was not. These results suggest that the activation of an IGF-1-dependent pathway by TES applied daily enables the regeneration of RGC axons in the ON, and that the prominent survival-promoting effects by TES applied daily may be related to IGF-1-independent pathways.

Materials and Methods

Animals. Adult male Wistar rats (250–300 g) were used. All experimental procedures were performed in accordance with the institutional guidelines for laboratory animal care and treatment and with the standards for care and use of laboratory animals of the U. S. National Institutes of Health guidelines.

ON surgery. The rats were anesthetized with 7% chloral hydrate solution (400 mg/kg, intraperitoneally). The left ON was then exposed and crushed 2 mm behind the globe with jewelry forceps (Dumont #545, World Precision Instruments, Sarasota, FL, USA) for 10 s, as described previously.[15] Direct ophthalmoscopy verified that the retinal blood flow was not damaged.

Transcorneal Electrical Stimulation. TES was applied immediately after the ON crush as described previously,[9] via a contact electrode (Kyoto Contact, Kyoto, Japan) applied to the cornea. In addition to systemic anesthesia, a drop of 0.4% oxybuprocaine hydrochloride (Benoxil; Santen Pharmaceutical, Osaka, Japan) was applied as surface anesthesia before the electrode was placed. Biphasic rectangular current pulses (1 ms, 100 μA) at 20 Hz were delivered by an electrical stimulation system (stimulator, Master-8, A.M.P.I., Jerusalem, Israel; isolator, BSI-950, Dagan, Minneapolis, MN, USA). Immediately after the ON crush, TES was applied for 1 h to groups of rats according to four different protocols: a single application of TES immediately after crush (day 0); two applications, on days 0 and 7; four applications, on days 0, 4, 7, 10; and daily applications, on days 0–12. For the second and subsequent TES, animals were anesthetized with 7% chloral hydrate solution (400 mg/kg, intraperitoneally). The rats of a control group received sham stimulation in which the electrodes were applied daily to their corneas for 1 h without any current.

Anterograde Labeling of RGC Axons. On day 12 after the ON crush, cholera toxin B subunit [CTB, 2.5 μg/μl in 2 μl phosphate-buffered saline (PBS); List Biological Laboratories, Campbell, CA, USA] was injected into the vitreous body to anterogradely label the RGC axons as described.[15] Two days after CTB application, the animals were transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) and 16-μm-thick longitudinal cryosections of the ON were made. CTB immunostaining was then performed as described.[15] After blocking, the sections were incubated in a solution of goat anti-CTB antibody (List Biological Laboratories) overnight at 4°C. After washing in PBS, the sections were incubated with a biotinylated rabbit anti-goat IgG antibody (Vector Laboratories, Burlingame, CA, USA) for 2 h at room temperature, followed by 1:400 Alexa 488-conjugated streptavidin (Molecular Probes, Eugene, OR, USA). Anterogradely labeled axons were observed with a fluorescence microscope (Optishot; Nikon, Tokyo, Japan), and confocal microscopic images were taken with a Zeiss LSM510 META instrument (Carl Zeiss, Göttingen, Germany).

Quantification of Axonal Growth. Axons were labeled as described.[15] Under a fluorescence microscope (Optishot) with ×200 magnification, the CTB-labeled axons on grid lines at 250, 500, or 1000 μm from the distal end of the crush site were directly counted. The mean number of axons at each distance was calculated for seven to ten sections per animal. These data are reported as means ± standard error of the mean. Statistical analysis for multiple comparison was performed by Scheffe's test.

Quantification of the RGC Density. RGCs were retrogradely labeled by placing a small sponge that had been soaked in 2% FluoroGold (FG; Fluorochrome, Englewood, NJ, USA) on both superior colliculi after craniotomy.[15–17] Seven days after FG application, the left ON was crushed with jewelry forceps. Twelve days later, each operated rat

was perfused transcardially with saline followed by 4% PFA in 0.1 M PB. The retinas were dissected and flat-mounted on glass slides, which were examined under a fluorescence microscope (E800; Nikon) with a UV filter (365 nm). The number of FG-labeled neurons was counted in 12 areas (1 mm^2 each) at distances of 1, 2, and 3 mm from the optic disc along the nasotemporal and dorsoventral midlines (upper, lower, nasal, and temporal directions). From the number of FG-labeled neurons counted in the 12 areas, the density of surviving RGCs was calculated. The data of RGC density are reported as means ± standard deviation. The statistical significance of differences was determined by one-way analysis of variance (ANOVA) followed by the Scheffe's test with significance set at $P < 0.05$.

IGF-1 Immunostaining of Retinal Sections. To examine localization of IGF-1 in the retina, immunohistochemistry of IGF-1 was performed as described.[9] After blocking of nonspecific background staining, vertical sections of the retina were immunolabeled with a mouse anti-IGF-1 antibody (×1000, Upstate Biotechnology, Lake Placid, NY, USA) overnight at 4ºC. After washing, the sections were treated with a proper secondary antibody conjugated with either Alexa 488 or 568 (×200, Molecular Probes) and incubated for 2 h at room temperature. Müller cells were immunostained with rabbit anti-glutamine synthase (GS) antibody (×200, Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Application of IGF-1 Receptor Antagonist. To examine the involvement of IGF-1 in axonal regeneration and survival promotion induced by TES, an IGF-1 receptor antagonist, JB3, was systemically administered. JB3 is a cyclic D-amino acid peptide analog of the D domain of IGF-1 (CSKAPPKLPAAYC)[18] and is synthesized nonbiologically (Sigma Genosys, Hokkaido, Japan). As a control for the sequence and conformational specificity of JB3, JB4, a scrambled version of the L-amino acid analog (CPKLYASPAAYC) was used. JB3 (100 or 200 µg/kg) and JB4 (100 µg/kg) were intraperitoneally injected immediately before each TES application. The JB3 dose was determined by reference to a previous report.[9] Regenerating axons or surviving RGCs were quantified in the same manner as described above.

Results

ON Regeneration Induced by TES

In the crushed ON without any application of TES or with sham stimulation, few CTB-labeled axons passed the crush site (Fig. 1A, B). However, the more frequent TES applications became, the more the number of axons passing through the crush site increased (Fig. 1C–F). A particularly large

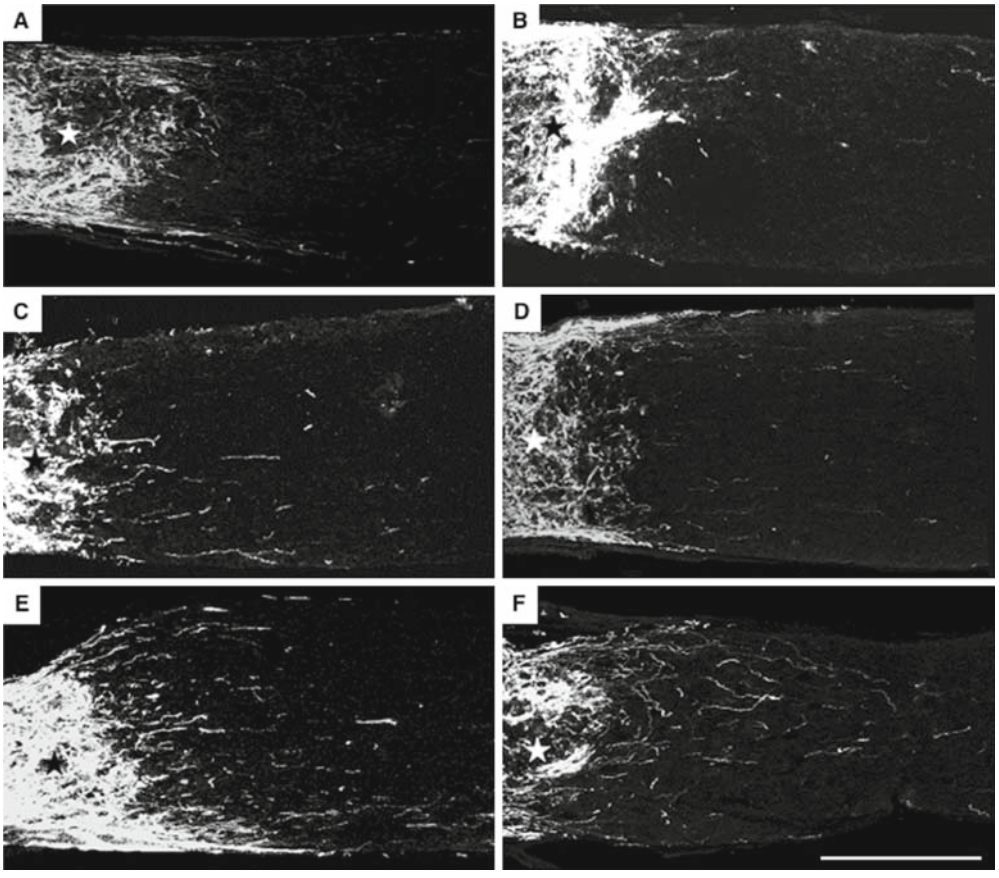

Figure 1A–F. Transcorneal electrical stimulation (TES) of axon regeneration in the crushed optic nerve. Regenerated axons in the optic nerve (ON) with crush only (**A**), with sham stimulation (**B**), with a single TES application (**C**), with two TES applications (**D**), with four applications (**E**), and with daily application (**F**), all labeled with cholera toxin B subunit (CTB). Asterisks indicate the crush site. The CTB-labeled axons extending through the lesion site increased gradually with the number of TES applications. Bar = 250 µm.

number of CTB-labeled axons was observed in the ON distal to the crush site in eyes with daily application (Fig. 1F). The quantitative data showed that the mean number of CTB-labeled axons gradually increased at the 250-μm measurement point as the number of TES application increased. The mean number by group was 2.06 ± 0.25 (crush only, $n = 13$), 2.61 ± 0.28 (sham stimulation, $n = 10$), 3.94 ± 0.54 (single TES, $n = 10$), 4.25 ± 1.19 (two applications of TES, $n = 9$), 5.18 ± 0.98 (four applications of TES, $n = 10$), and 5.94 ± 0.92 (TES applied daily, $n = 9$). A significant difference was observed between sham stimulation and daily stimulation ($P < 0.05$, Scheffe's test). At the 500- and 1000-μm measurement points, the mean number of CTB-labeled axons did not significantly increase for any frequency of TES application (Fig 2, middle and bottom). These results indicate that TES promoted regeneration of RGC axons within a distance of 250 μm of the crush site, and the regeneration gradually increased as the number of TES applications increased.

Upregulation of Retinal IGF-1 Expression by TES Applied Daily

A previous study revealed that TES upregulated retinal IGF-1 expression in the intact rat eye.[9] We therefore studied how retinal IGF-1 levels were changed by a single or daily application of TES after an ON crush. In the normal rat retina, only slight immunoreactivity of IGF-1 was apparent in the ganglion cell layer (GCL) and the outer plexiform layer (OPL) (Fig. 3A). However, 3 days after the ON crush, retinal levels of IGF-1 immunoreactivity increased (Fig. 3B), and both a single and daily applications of TES increased IGF-1 immunoreactivity in the GCL, OPL, and inner nuclear layer (INL) (Fig. 3E, H). The IGF-1 immunostaining intensity in the retina without TES fell on day 7 and returned to normal levels by day 12 (Fig. 3C, D). At the same time, immunoreactivity in the retinas treated with a single TES application also returned to normal levels (Fig. 3F, G). However, TES applied daily seemed to increase IGF-1 in the GCL on day 7 (Fig. 3I), with the highest intensity reached on day 12, not only in the GCL but also in the INL and OPL (Fig. 3J). Double-staining of IGF-1 and glutamine synthase (GS) showed that immunoreactivity of IGF-1 merged partially with that of GS (Fig. 3K–M). These results indicate that with daily TES applications, retinal IGF-1 continued to be upregulated in the whole retinal layer until day 12, which was not the case with the single application.

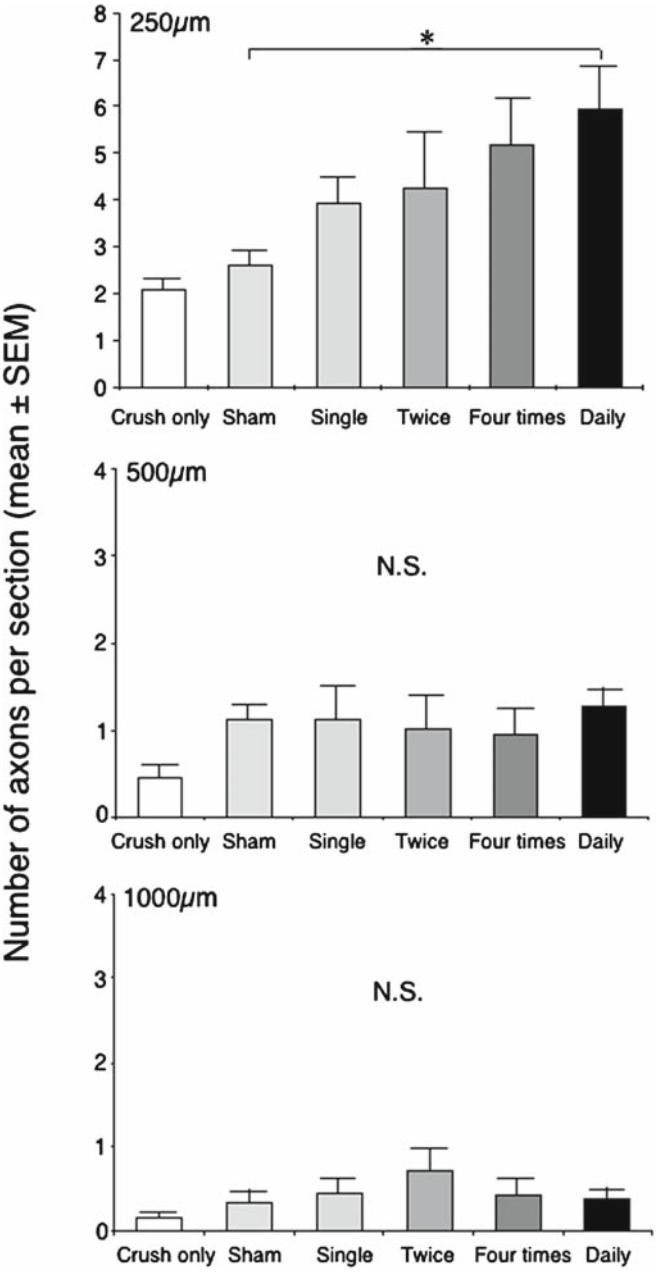

Figure 2. Quantitative analysis of CTB-labeled regenerating axons in each TES group. At 250 μm distance from the lesion site, daily applications of TES significantly increased the number of CTB-labeled axons. No significant difference, compared with sham stimulation, was detected in any TES group at either 500 or 1000 μm. Statistical analysis was performed by one-way analysis of variance (ANOVA) and Scheffe's test for multiple comparisons. *$P < 0.05$.

Inhibition of TES-Induced Axonal Regeneration by the IGF-1 Receptor Antagonist

To reveal the relationship between the axonal regeneration induced by TES and the upregulation of retinal IGF-1, the IGF-1 receptor antagonist JB3 was administered to see whether it blocked axonal regeneration by daily applications of TES. First, the extension of many RGC axons at the crush site proximal to the center of the ON treated with daily applications of TES was confirmed (Fig. 4A). This regrowth-promoting effect of the daily application of TES was suppressed by JB3 administration before each TES, and CTB-labeled axons were scarcely observed in the portion distal from the crush site (Fig. 4B). When JB4, a nonfunctional analog of JB3, was systemically administered

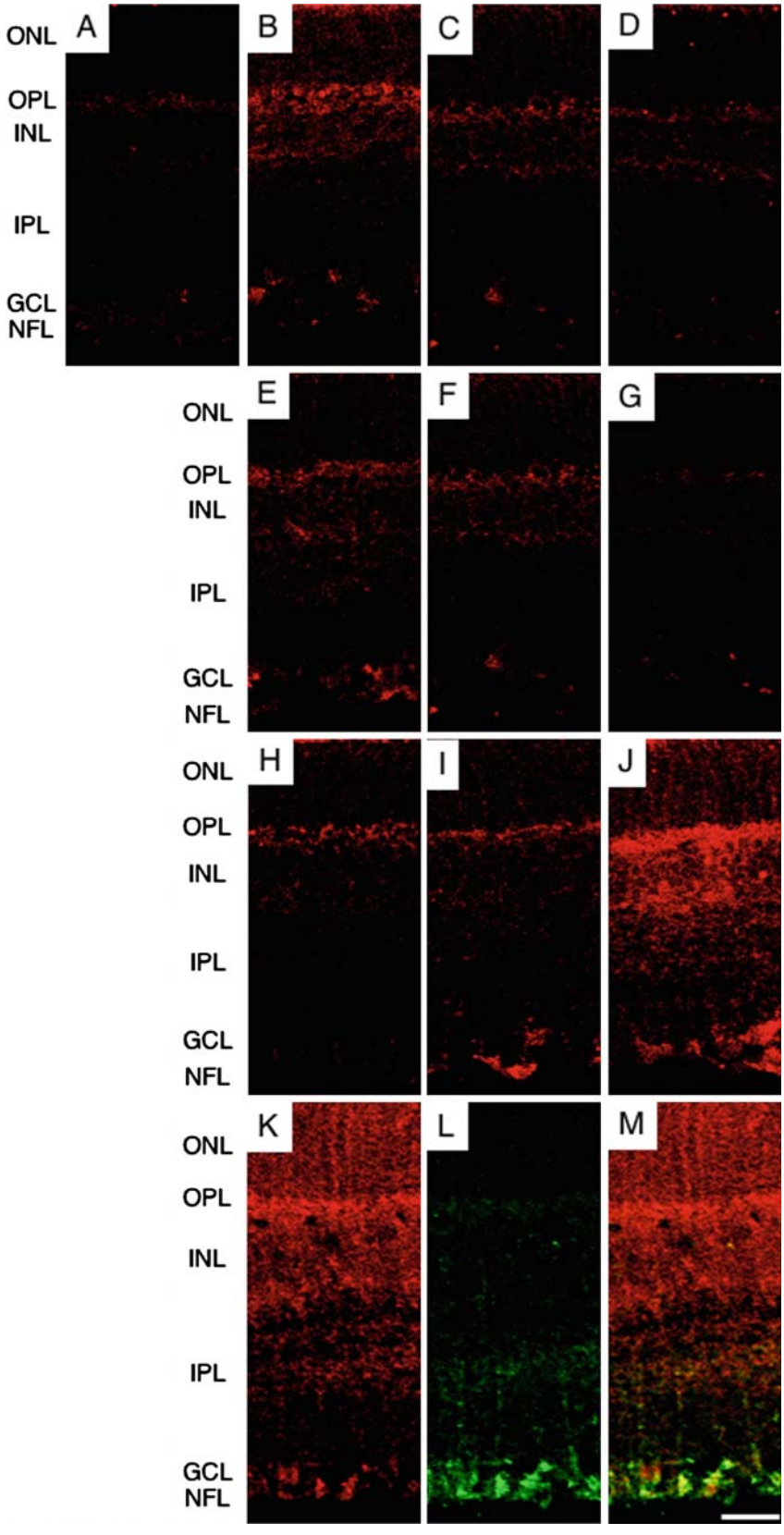

Figure 3A–M. Immunohistochemistry of insulin-like growth factor 1 (IGF-1) in the retina after ON crush with TES. In the normal retina (**A**), slight IGF-1 immunoreactivity was detected in the ganglion cell layer (*GCL*) and outer plexiform layer (*OPL*). On day 3, IGF-1 immunoreactivity increased in the retinal ganglion cell (RGC) OPL and inner nuclear layer (*INL*) of the retina with crush only (**B**), and with a single (**E**) or a daily (**H**) application of TES. On day 7, in the retina with crush only (**C**) or a single application of TES (**F**), IGF-1 immunoreactivity decreased, and on day 12 it returned to normal (**D, G**). However, in the retina with daily application of TES, IGF-1 expression gradually increased in the GCL, INL, OPL, and Müller cells (**I** day 7; **J** day 12). In the double staining on day 12 with daily application of TES, immunoreactivity of glutamine synthase (GS; **L**) was colocalized with IGF-1 (**K, M**, merged). Bar = 20 µm. *ONL*, outer nuclear layer; *IPL*, inner plexiform layer; *NFL*, nerve fiber layer.

Jpn J Ophthalmol
Vol 53: 257–266, 2009

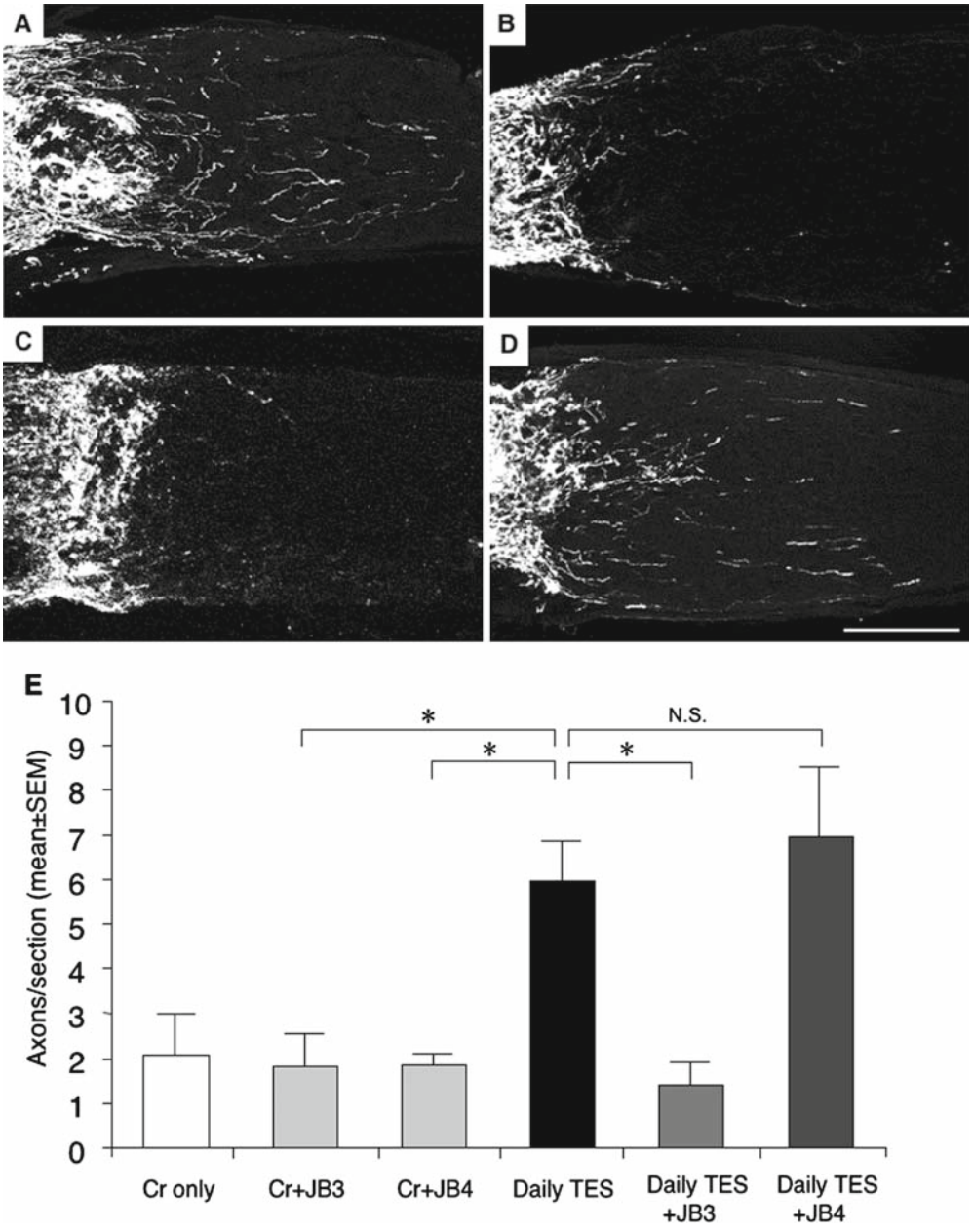

Figure 4A–E. Inhibition of axonal regeneration by the IGF-1 receptor antagonist. In the crushed ON with daily application of TES (**A**), many CTB-labeled axons passed the crush site, whereas with daily application of TES + JB3, IGF-1 receptor antagonist (**B**) markedly inhibited the regrowth of RGC axons. In the ON with only JB4, a nonfunctional counterpart of JB3 (**C**), there were few axons at sites distal from the crush injury. In the ON with daily application of TES + JB4 (**D**), many axons extended, passing through the crush site. Quantitative analysis of CTB-labeled axons at 250 μm from the crush site (**E**) showed that there was a significant difference between daily application of TES and the daily application of TES combined with JB3 administration. Data are means ± SEM. Statistical analysis was performed by one-way ANOVA and Scheffe's test for multiple comparisons (*$P < 0.05$). *Cr*, crush. Bar = 250 μm.

instead of JB3, the axonal regeneration induced by the TES was not inhibited (Fig. 4D). Quantitative data at 250 μm distance showed that the mean number of CTB-labeled axons with daily application of TES combined with JB3 administration (1.93 ± 0.67, $n = 6$) significantly decreased, compared with daily application of TES alone (5.94 ± 0.92, $n = 9$) (Fig. 4E). The mean number of labeled axons with daily injection of JB4 and TES was 6.94 ± 1.57 ($n = 5$), and was not significantly different from that with daily application of TES alone. These results indicate that the administration of the IGF-1 receptor antagonist inhibited the regeneration of ON axons induced by daily application of TES, suggesting that axonal regeneration by daily application of TES is mediated by IGF-1 receptors.

Survival-Promoting Effect Induced by TES Applied Daily Was Not Attenuated by the IGF-1 Receptor Antagonist

Because the axon-regenerative effects of TES applied daily were limited to a relatively short distance, they could have resulted from the promotion of RGC survival. Therefore, the survival-promoting effects between a single TES application and TES applied daily were compared, and the possibility that the application of JB3 might inhibit the regeneration was investigated. As well as the evaluation of axonal regeneration, JB3 was applied before each TES. In normal retinas, FG-labeled RGCs can be recognized by a fine-dotted pattern of fluorescence in the perinuclear cyto-

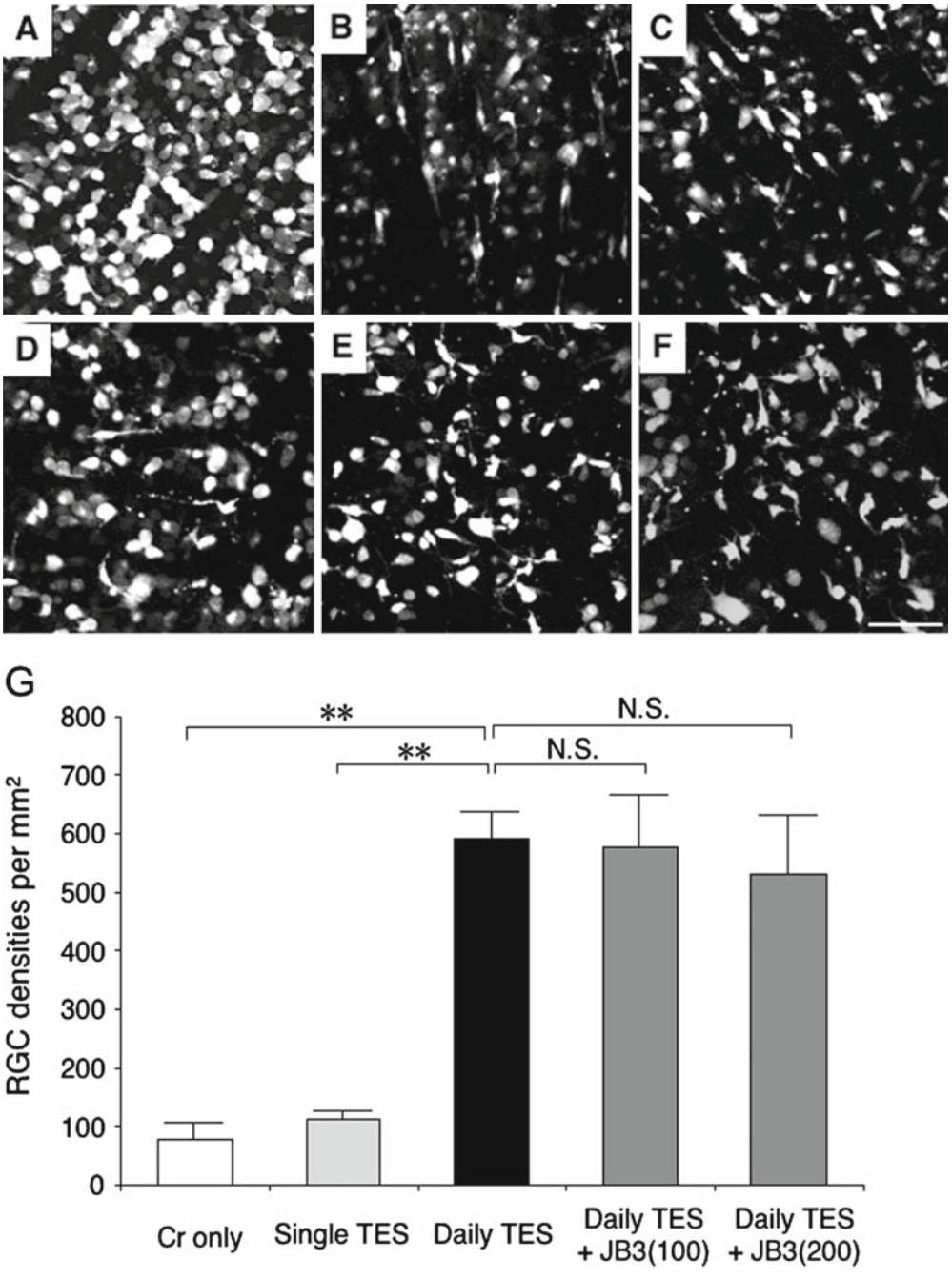

Figure 5A–G. Survival-promoting effect by daily application of TES was independent of the IGF-1 pathway. Photomicrographs of surviving RGCs, retrogradely labeled with FluoroGold before ON crush, in the corresponding regions of a flat-mounted retina (1 mm from the optic disc) (**A–F**). The retina with crush only (**B**) or with a single application of TES (**C**) apparently had fewer labeled RGCs and more glial cells, which phagocytose the RGC debris, than the normal retina (**A**). The surviving RGCs in the retina with daily TES markedly increased (D). In the retina with daily TES and JB3 (100 μg/kg) (**E**), the surviving RGCs also increased. Even though a higher concentration of JB3 (200 μg/kg) was administered with TES, many surviving RGCs were observed as well as the retina with daily application of TES (**F**). The mean density of labeled RGCs with daily application of TES was significantly higher than that of those with crush only or with a single application of TES, whereas there was no significant difference in RGC density between daily application of TES and daily application of TES with JB3 (100 or 200 μg/kg) (**G**). The data are means ± SD of labeled RGCs/mm^2. Statistical analysis was performed by one-way ANOVA and Scheffe's test for multiple comparisons ($P < 0.01$). Bar = 50 μm.

plasm and proximal dendrites (Fig. 5A). On day 12 after the ON crush, we found a remarkable reduction in the number of FG-labeled RGCs, along with some irregularly shaped RGCs and dead RGC debris (Fig. 5B). A similar decrease was also found in the retinas with a single application of TES immediately after the ON crush (Fig. 5C), whereas many surviving RGCs were found in the retinas treated daily with TES (Fig. 5D), suggesting that a daily application of TES has a neuroprotective effect on RGCs 12 days after an ON crush as well as a regenerative effect on RGC axons. That neuroprotective effect was not counteracted by JB3 (100 μg/kg) application even at a higher concentration (200 μg/kg) (Fig. 5E, F). The mean density of FG-labeled RGCs on day 12 in retinas with crush only was 78 ± 25/mm^2 ($n = 6$), and that with a single application of TES was 112 ± 16/mm^2 ($n = 6$), whereas the mean density of FG-labeled RGCs with daily TES was 592 ± 46/mm^2 ($n = 6$). Daily application of TES significantly increased the mean density by 7.6-fold compared with crush only ($P < 0.01$), and it was significantly increased by 5.2-fold compared with a single TES application. The daily application of TES combined with JB3 (100 or 200 μg/kg) resulted in a mean density of 578 ± 20/mm^2 ($n = 6$), which was not significantly lower than that with the daily application of TES

alone (530 ± 99/mm^2, n = 5). These results show that daily applications of TES promoted the survival of RGCs with crushed axons, and that IGF-1 receptor was not much involved in this survival effect. Together with the previous findings that axonal regeneration by daily application of TES data is mediated by IGF-1 receptor, it is now clear that axonal regeneration by daily application of TES is not only a consequence of the enhancement of RGC survival but also an effect of other factors.

Discussion

The present study showed that TES promotes axonal regeneration after ON crush. The promotion of axonal regeneration correlated with the number of TES applications, and the daily application of TES was the most effective. Second, daily TES upregulated retinal IGF-1 expression more than a single application of TES did. Third, daily application of TES also significantly promoted the survival of RGCs after the crush. Finally, the axonal regeneration by the daily application of TES was completely blocked by a specific antagonist to IGF-1 receptor, whereas the promotion of RGC survival was not.

Axonal Regeneration by Daily TES

The present study showed that axonal regeneration by the daily application of TES after ON crush apparently upregulated retinal IGF-1, and that the regeneration was significantly blocked by an IGF-1 receptor antagonist. Thus, we suggest that activation of the retinal IGF-1 system is an important contributor to the axon-regenerating effect of daily applications of TES. Recent studies have suggested that the IGF-1 pathway contributes to axonal regeneration of RGCs in mammalian retinas. In adult rats, retinal IGF-1 levels begin to downregulate from day 3 after ON crush.[14] On the other hand, the retinal IGF-1 levels of goldfish are capable of inducing spontaneous ON regeneration, increase during days 1–3 after ON crush, peak on day 5, and then gradually decrease.[19] It has also been shown that in retinal explant cultures of adult rats, direct application of IGF-1 promotes the elongation of RGC axons through activation of the Akt-PI3K pathway.[14] These results support our conclusion that the axon regeneration promoted by a daily application of TES is dependent on activation of the IGF-1 pathway.

Survival-Promoting Effects of Daily TES

A previous study has shown that a single TES application immediately after ON transection promotes RGC survival on day 7, and this survival-promoting effect is significantly blocked by an IGF-1 receptor antagonist.[9] In the present study, a single application of TES had no effect on RGC survival on day 12 after the ON crush. Although the reasons are not clear as to why the results for a single application of TES differed between the two studies, the difference may be due to the different method used to induce the ON damage, crush or transection. Unlike a single application of TES, the daily application of TES markedly promoted RGC survival on day 12 after the ON crush and this survival effect was hardly inhibited by the IGF-1 receptor blocker. Thus, although the effect is not distinguishable from the enhancement of RGC survival, axonal regeneration is promoted by daily application of TES, which presumably activates both IGF-1-dependent and -independent pathways that promote both axonal regeneration and survival of RGCs.

It has recently become accepted that axonal regeneration is not simply a consequence of survival enhancement. For example, overexpression of the bcl-2 anti-apoptotic gene markedly promotes the survival of axotomized RGCs.[20–22] However, even if environments favorable to axonal regeneration, such as peripheral nerve grafts or neutralizing CNS myelin with IN-1 antibodies, are available to the injured ON of bcl-2-overexpression mice, the number of regenerating axons does not increase in comparison with wild-type mice.[21,22] Along this line of evidence, the present results clearly indicate that axonal regeneration and survival of adult retinal ganglion cells are regulated by different mechanisms.

TES as a Novel Treatment for ON Regeneration

Many attempts have been made to promote axonal regeneration since it was revealed that ON axons can regenerate for a long time under favorable circumstances such as a peripheral nerve graft.[23,24] Short but robust regeneration of RGC axons has been achieved in crushed optic nerves by using an angiotensin II inhibitor,[25] *Clostridium botulinum* C3 enzyme to inactivate RhoA,[26] an inhibitor of Rho kinase,[27] induction of an inflammatory response,[28–30] and the transplantation of olfactory ensheathing cells with the potential to regenerate axons.[31] In comparison with these findings, daily application of TES does not facilitate axonal regeneration as robustly, and its effect is limited to a relatively short distance from the crush site. However, TES has a great advantage because it has already been applied to human ONs and retinal vascular diseases without any major complications,[32,33] and the noninvasive application of a brief ES may lead to axonal regeneration without the need for a surgical procedure. To improve the axon-regenerating effect, increasing the continuity of the electrical stimulation is likely to be a more effective strategy for extending the regenerated axons of RGCs for longer periods. Further experiments are needed to develop the necessary devices to deliver continuous ES, because daily applications of TES for a long time are harmful to the cornea. Furthermore, the possibility exists for a combined strategy applying the various treatments mentioned above, especially treatment with neurotrophic factors, because it has been demonstrated in vitro that electrical stimulation in combination with several neurotrophic factors promotes neurite outgrowth.[6]

Neuroprotection of TES in Human Eye Disease

TES has been clinically applied to the treatment of retinal and ON diseases, and it has been demonstrated that visual deterioration of ischemic and traumatic optic neuropathy and retinal artery occlusion are ameliorated by TES.[32,33] A previous report revealed that TES promotes the survival of degenerated photoreceptors in RCS rats, an animal model of retinitis pigmentosa.[10] The present study revealed that daily application of TES is much more effective for RGC survival than a single application of TES. Taking these results into account, chronic repetitive electrical stimulation of the eye might become the effective strategy of choice to improve visual performance not only in ON diseases but also retinal degenerative diseases.

Conclusions

The results of the present study suggest that the mechanisms for the survival and for the axonal-promoting effects of RGCs by daily application of TES are different. This is an issue that needs more research but which might provide a new avenue for understanding what regulates the survival of axonal regeneration of RGCs, and how different intracellular signaling happens whenever the neurons are activated by electrical stimulation in vivo. Investigation of the exact underlying mechanism of each novel effect by repetitive electrical stimulation is planned.

Acknowledgments. This work was supported by a Grant-in-Aid for Researchers, Hyogo College of Medicine, 2006.

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