PROSTAGLANDIN E1 ALLEVIATES NEUROPATHIC PAIN AND NEURAL DYSFUNCTION FROM ENTRAPMENT NEUROPATHY ASSOCIATED WITH DIABETES MELLITUS

TADAHIRO NATSUME, M.D.,* KATSUYUKI IWATSUKI, M.D., TAKANOBU NISHIZUKA, M.D., TETSUYA ARAI, M.D., MICHIRO YAMAMOTO, M.D., Ph.D., and HITOSHI HIRATA, M.D., Ph.D.

In this report, we present the results of investigation of the effects of prostaglandin E1 (PGE1) on entrapment neuropathy using a diabetic rat. A total of 60 male Sprague-Dawley rats were used in the study. The model of tibial nerve entrapment neuropathy associated with diabetes mellitus was created by streptozotocin-induced diabetic rats reared in cages with wire grid flooring. Rats were assigned to four groups: nondiabetic ($n = 15$), untreated diabetic ($n = 15$), diabetic treated with 30 μg/kg PGE1 ($n = 15$), and diabetic treated with 100 μg/kg PGE1 ($n = 15$). Pain tests and electrophysiological tests were performed at 0, 2, and 4 weeks, and assessments of gait, histology, and mRNA expression levels were performed at 4 weeks after initiating the PGE1 administration. In the 30 and 100 μg groups, the mechanical withdrawal thresholds measured by pain tests at 4 weeks (36.2 ± 16.4 g and 31.7 ± 15.3 g, respectively) and the motor conduction velocity (24.0 ± 0.2 m/s and 24.4 ± 0.3 m/s, respectively) were significantly higher than the untreated diabetic group (all $P < 0.05$) and lower than the nondiabetic group (all $P < 0.001$). In the gait analysis, the mean intensities in the 30 and 100 μg group (128.0 ± 20.1 a.u. and 109.0 ± 27.8 a.u., respectively) were significantly higher than the untreated diabetic ($P < 0.01$) and were not significantly different from the nondiabetic group ($P = 0.81$). Fiber density ($P = 0.46$) and fiber diameter ($P = 0.15$) did not show any significant differences. PGE1 significantly decreased nerve growth factor (NGF) mRNA and increased vascular endothelial growth factor (VEGF) mRNA in the tibial nerve (both $P < 0.01$). In conclusion, neurological deteriorations of diabetic rats were alleviated with PGE1, which is associated with inhibition of NGF and enhancement of VEGF at the entrapment site. © 2014 Wiley Periodicals, Inc. Microsurgery 00:000–000, 2014.

Chronic compression or strain on a nerve from prolonged or repeated external force, ischemia, and scaring in and around the nerve are the three major contributing factors to the development and progression of entrapment neuropathies.[1] Some conditions cause nerves particularly susceptible to compression. These include diabetes mellitus in which nerve circulation is already compromised.[2,3]

Entrapment neuropathies are highly prevalent in the diabetic population with a reported prevalence of more than 30%.[4] Entrapment neuropathies occur not only in the upper extremities but also in the lower extremities. Surgical decompression is definitely a viable option for entrapment neuropathies in the extremities that are associated with diabetes mellitus,[5,6] and surgery has been demonstrated to provide significant pain relief and prevention of ulceration and subsequent amputation in many of these patients.[7–9] However, the effect of treatment is not consistent. Development of an adjuvant treatment to reinforce the effect of the surgery on nerve recovery is highly desirable.

Prostaglandin E1 (PGE1) is a drug that is widely used for treating critical limb ischemia. The mechanism of action of PGE1 has been reported to be via its activity in dilating the peripheral blood vessels and increasing their blood flow. PGE1 may have the potential to help recovery from entrapment neuropathy in diabetics.[10] It is thought that an activity other than the blood flow-improving activity may be involved in its action mechanism.

Zochdne et al.[11] studied a distal tibial mononeuropathy that involved hind foot of diabetic rats reared in cages with wire grid flooring. This appears to be a useful animal model of entrapment neuropathy in diabetics. The purpose of the current study was to investigate the effects of PGE1 on entrapment neuropathy in diabetics and to determine the mechanisms of neuropathic pain and neural dysfunction in these settings using the model proposed by Zodchone et al.

MATERIALS AND METHODS

Animal Models

All experimental protocols and animal maintenance procedures performed in this study were approved by the Animal Ethics Research Committee of Nagoya University. A total of 60 male Sprague-Dawley rats (SLC, Japan), aged 7 weeks old, were used in this experiment. Forty-five rats received a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ) dissolved in citrate buffer (pH 4.5) to induce diabetes. Nondiabetic rats received an equivalent volume of the citrate buffered solution. Hyperglycemia was verified 1 week after the STZ injection by blood sampling from the tail vein. Whole-blood glucose tests were performed by cutting the

Department of Hand Surgery, Nagoya University Graduate School of Medicine, Showa-Ku, Nagoya-Shi, Japan

*Correspondence to: Tadahiro Natsume, M.D., Department of Hand Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-Cho, Showa-Ku, Nagoya-Shi 466-8560, Japan. E-mail: tadahiro_natsume@kuc.biglobe.ne.jp

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top of the caudal vein and by assessing glucose levels with a ONE TOUCH Ultra glucometer (Johnson & Johnson). A fasting blood glucose level of $\geq$300 mg/dl was considered to indicate experimental diabetes.[12] In diabetic rats, PGE1 was administered via gastric gavage at a dose of ~2 ml (30 or 100 μg/kg) twice per day for 4 weeks. The same volume of saline was given in a similar manner to control rats. The animals were housed in a temperature-controlled environment and maintained on a 12-h light–dark cycle with food and water provided ad libitum. Rats were randomly assigned to the following four groups: nondiabetic rats (hereinafter referred to as normal rats; $n = 15$); STZ-induced untreated diabetic rats (hereinafter referred to as control rats; $n = 15$); STZ-induced diabetic rats treated with 30 μg/kg PGE1 ($n = 15$); and STZ-induced diabetic rats treated with 100 μg/kg PGE1 ($n = 15$).

Behavioral Testing

Mechanical allodynia assessments (von Frey hair test). To assess mechanical allodynia, we performed the von Frey hair test. The paw withdrawal threshold in response to a mechanical stimulus was determined by using a series of filaments of varying thickness. We used the Touched-Test von Frey filaments (North Coast Medical, CA), ranging from 0.16 to 100 g. A cutoff of 100 g of hair was selected as the upper limit for testing, as stiffer hairs tended to raise the entire limb rather than buckle, substantially changing the nature of the stimulus. Animals were placed in a plastic cage with a metal mesh floor and were allowed to move around the cage freely for ~10 min prior to testing to ensure that they were acclimatized to the environment. Probing of the paws was only performed when the four paws of the rat were in contact with the floor. von Frey filaments were applied to the mid-plantar surface of both hind paws to induce slight buckling against the paw and held for approximately 6–8 seconds.[13] Stimuli were presented at intervals of several seconds, allowing for apparent resolution of any behavioral responses to the previous stimuli. A minimum of three withdrawals of the tested paw of five filament applications was considered to indicate a positive response. Filaments were applied in ascending order, and the smallest filament that elicited a foot withdrawal response was considered the threshold stimulus. The von Frey hair tests were performed at 0, 2, and 4 weeks after initiating the PGE1 administration (five rats were examined in each group at each check point). The first assessment was carried out on day 0 to determine the baseline threshold for each group.

Gait analysis. A detailed gait analysis was performed on walking rats using the CatWalk according to the company's instruction (Noldus, Wageningen, Netherlands).[14] Briefly, light from a fluorescent tube was sent through a glass plate, and the light rays were completely reflected internally. As soon as the rat's paw came in contact with the glass surface, the light was reflected downward. This resulted in a sharp image of a bright paw print. The whole run was recorded via a camera placed under the glass plate. The intensity of the paw print and the tibial functional index (TFI) were measured. The intensity of the paw print was expressed in arbitrary units (a.u.); this parameter reflected the mean pressure exerted by an individual paw during floor contact and the whole crossing of the walkway. Data analysis was performed with the threshold value set at 40 a.u. (possible range 0–225), or more specifically, all pixels brighter than 40 were used. Gabriel et al.[14] suggested that there is a correlation between the development of mechanical allodynia and intensity of the paw print. Thus, we determined the intensity of the paw print to objectively assess mechanical allodynia.

The TFI was a weighted index of function to evaluate the impairment in gait resulting from tibial nerve lesions.[15] The tracks were evaluated for three different parameters: toe spread, which is the distance between the first and fifth toes; intermediate toe spread, which is the distance between the second and fourth toes; and print length, which is the distance between the third toe and the hind pad. The TFI is a negative indicator of the degree of nerve dysfunction varying from zero to −100, with zero corresponding to normal function and −100 indicating complete dysfunction.[15] These parameters were measured from the footprints of normal and diabetic rats. This technique has been shown to give highly reproducible results. The gait analysis was performed at 4 weeks after initiating the PGE1 administration (five rats were examined in each group).

Electrophysiological Examinations

At 0, 2, and 4 weeks after initiating the PGE1 administration, sciatic nerves were exposed, and electrophysiological recordings were obtained under anesthesia (five rats were examined in each group at each check point). The first assessment was carried out on day 0 to determine the baseline for each group. Anesthesia was induced by inhalation of 5% isoflurane, which was maintained at a concentration of 2–3%, as needed. For sciatic–tibial studies, we recorded the signal from the dorsal subcutaneous space of the hind foot using a platinum electrode (H537A; Nihon Koden, Tokyo, Japan), which was stimulated by a bipolar stimulating electrode (UM2-5050; Nihon Koden) located ~7.0 cm proximal to the recording point. Electrical pulses (supramaximal; duration of 100 ms; frequency of 1 Hz; and square wave) were applied with an isolator (SS-201J; Nihon Koden) connected to an electronic stimulator. The motor conduction

velocity (MCV) was calculated based on the latency and the distance between the stimulating and recording electrodes. MCV was recorded to estimate the electrophysiological function. During the recordings, the temperature near the nerve was maintained at 37°C with a heat lamp. Rats were sacrificed after electrophysiological examinations.

Histological Examinations

At the end of the experiment (i.e., 4 weeks after initiating PGE1 administration) and after completing the von Frey hair test, gait analysis, and electrophysiological testing, animals were sacrificed, and tibial nerves from the middle of the tarsal tunnel were quickly harvested (five rats were examined in each group). The nerve was excised and fixed in 2.5% glutaraldehyde. For postfixation, 1% osmic acid was used. The tissues were then dehydrated through an ascending series of ethanol solutions and embedded in epon. Transverse semithin sections (~1 μm thick) of the tibial nerves were stained with toluidine blue and used for morphometric analysis of myelinated fiber density and fiber diameter. Myelinated fiber density and diameter were calculated from photomicrographs of the transverse sections of nerve fascicles at a final magnification of 400× and measured using an image-analyzing system (Biozero BZ-8000; KEYENCE, Osaka, Japan).

mRNA Expression

With the same rats as histological examination, tibial nerves from the middle of the tarsal tunnel and dorsal root ganglion (DRG) L5 were harvested (five rats were examined in each group). These were immediately frozen in liquid nitrogen and stored at −80°C prior to the preparation of RNA. Total RNA was harvested, reverse transcribed, and assessed with real-time polymerase chain reaction to determine the expression of nerve growth factor (NGF) mRNA in the tibial nerve and DRG and of vascular endothelial growth factor (VEGF) mRNA in the tibial nerve. The real-time reactions were performed using the Taqman Gene Expression Assay (Applied Biosystems, Foster City, CA) on an AB StepOne Real-time PCR System (Applied Biosystems). For each gene, a set of primers and a probe were chosen from the Applied Biosystems list of TaqMan® Gene Expression Assays. The expression of β-actin was used as an endogenous control for normalization, and the nontransduced samples were used as calibrator controls. Data were then collected via the AB StepOne Real-Time PCR System and analyzed with the comparative C_T method using the SDS version 1.3.1 Relative Quantification software.

Statistical Analysis

All data are presented as the mean ± standard deviation. A two-way repeated-measures analysis of variance (ANOVA) was performed to determine the differences in MCV and withdrawal threshold, as measured by the von Frey hair test, between each week in all groups. A one-way ANOVA followed by a Bonferroni multiple comparison test was also performed to determine the differences in the intensity of the paw prints and TFI, histological examination, and mRNA expression levels at 4 weeks in all groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Clinical Observations

A diabetic rat treated with 30 μg PGE1 died during preparation for the second week assessment. This rat was excluded from the analysis. All diabetic rats had a marked impairment of growth. At the beginning of the experiment, body weight was not significantly different among the four groups ($P = 0.65$), and their mean body weight was 248.5 ± 18.7 g. Four weeks later, the rats that had received citrate buffer alone weighed 395.5 ± 8.1 g, whereas those given STZ weighed only 282.1 ± 59.7 g. Rats given STZ in combination with PGE1 weighed 272.6 ± 51.6 g (the 30 μg PGE1-treated group) and 265.8 ± 42.8 g (the 100 μg PGE1-treated group). Normal rats were significantly heavier when compared with the diabetic rats ($P < 0.001$). Body weight was not significantly different among the diabetic rats ($P = 0.85$). All diabetic rats exhibited marked hyperglycemia. Glucose levels at 4 weeks in normal rats were significantly lower when compared with the diabetic rats (149.8 ± 20.4 mg/dl in normal rats; 398.2 ± 47.2 mg/dl in control rats; 432.2 ± 52.3 mg/dl in the 30 μg PGE1-treated diabetic rats; and 413.2 ± 42.5 mg/dl in the 100 μg PGE1-treated diabetic rats; $P < 0.001$). Glucose levels at 4 weeks were not significantly different among the diabetic rats ($P = 0.75$).

Mechanical Allodynia Assessments (von Frey Hair Test)

The withdrawal threshold at baseline was not significantly different among the four groups. All diabetic rats (i.e., control and the 30 μg PGE1-treated group and the 100 μg PGE1-treated group) displayed a progressive and significant reduction in mechanical nociceptive threshold in each examination. In the normal group, the withdrawal threshold at 2 and 4 weeks was 56.8 ± 39.2 g and 72.6 ± 25.8 g, respectively, and these were significantly higher than those of the control group (2 weeks: 22.7 ± 5.3 g; 4 weeks: 19.1 ± 7.8 g; $P < 0.001$). The mechanical withdrawal thresholds at 2 weeks in the 30 μg PGE1-treated group (27.2 ± 12.4 g) and the 100 μg PGE1-treated group (24.9 ± 3.5 g) were not significantly

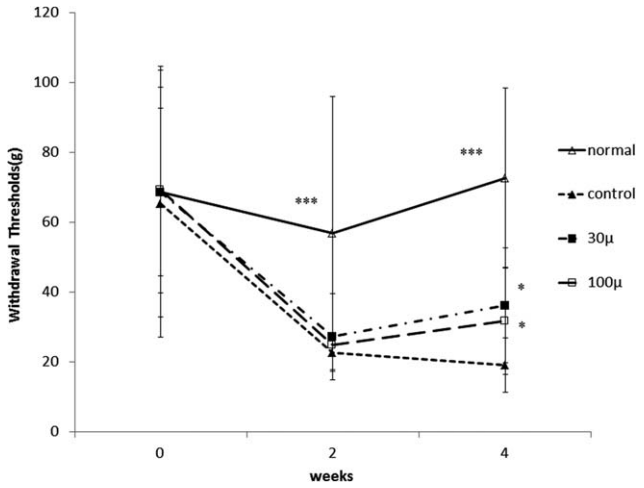

*Figure 1. The mechanical withdrawal thresholds, as determined via the von Frey hair test, at 0, 2, and 4 weeks after prostaglandin E1 (PGE1) administration. The mechanical withdrawal threshold was significantly lower in the control group when compared with the normal group at 2 and 4 weeks ($P < 0.001$). The mechanical withdrawal threshold was significantly higher in the PGE1-treated groups when compared with the control group at 4 weeks ($P < 0.05$). *$P < 0.05$ and ***$P < 0.001$ vs. control rats.*

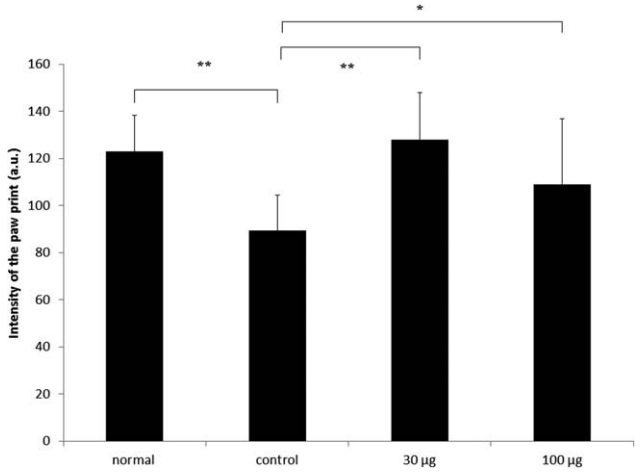

*Figure 2. Mean intensity of paw placement, a more objective assessment of mechanical allodynia, was determined via the CatWalk analysis at 4 weeks after prostaglandin E1 (PGE1) administration. The mean intensity was significantly lower in the control group when compared with the normal group ($P < 0.01$). The mean intensity was significantly higher in the PGE1-treated groups when compared with the control group (30 μg PGE1 group: $P < 0.01$; 100 μg PGE1 group: $P < 0.05$). *$P < 0.05$ and **$P < 0.01$ vs. control rats.*

different from that of the control group at 2 weeks (22.7 ± 5.3 g; $P = 0.48$).

The mechanical withdrawal thresholds at 4 weeks in the PGE1-treated groups (30 μg group: 36.2 ± 16.4 g; 100 μg group: 31.7 ± 15.3 g) were significantly lower than that of the normal group (72.6 ± 25.8 g; $P < 0.001$). The mechanical withdrawal thresholds at 4 weeks in the

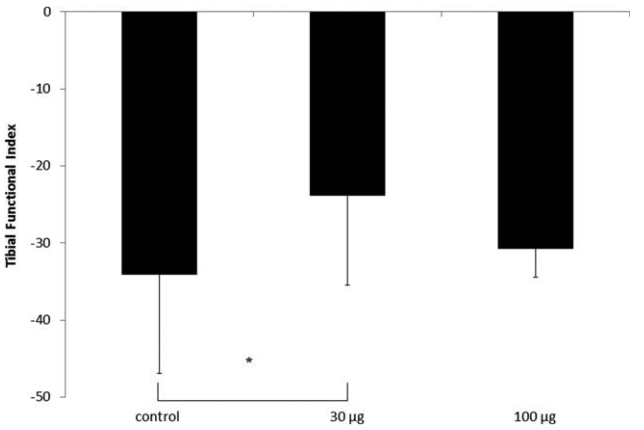

*Figure 3. The tibial functional index (TFI), an assessment of tibial nerve recovery in rats, was determined via CatWalk analysis at 4 weeks after prostaglandin E1 (PGE1) administration. TFI in the 30 μg PGE1 group was significantly higher versus the control group ($P < 0.05$). There were no significant differences between the 100 μg PGE1 and control groups ($P = 0.36$). *$P < 0.05$ vs. control rats.*

PGE1-treated groups (30 μg group: 36.2 ± 16.4 g; 100 μg group: 31.7 ± 15.3 g) were significantly higher than that of the control group at 4 weeks (19.1 ± 7.8 g; $P < 0.05$; Fig. 1).

Gait Analysis

After 4 weeks of PGE1 administration, the mean intensity in the control group (89.6 ± 14.8 a.u.) was significantly lower than that of the normal group (123.0 ± 15.3 a.u.; $P < 0.01$). In the 30 μg PGE1-treated group and the 100 μg PGE1-treated group, the mean intensity (30 μg group: 128.0 ± 20.1 a.u.; 100 μg group: 109.0 ± 27.8 a.u.) was significantly higher than that of the control group (89.6 ± 14.8 a.u.; $P < 0.01$). The mean intensity of PGE1-treated groups (30 μg group: 128.0 ± 20.1 a.u.; 100 μg group: 109.0 ± 27.8 a.u.) at 4 weeks were not significantly different from that of the normal group (123.0 ± 15.3 a.u.; $P = 0.81$; Fig. 2).

After 4 weeks of PGE1 administration, TFI in the 30 μg PGE1-treated group (-23.8 ± 11.7) was significantly higher than that of the control group (-34.1 ± 12.8; $P < 0.05$). TFI in the 100 μg PGE1-treated group (-30.7 ± 3.7) was not significantly higher than that of the control group ($P = 0.36$; Fig. 3).

Electrophysiological Examinations

MCV at baseline was not significantly different among the four groups (normal group: 24.1 ± 1.2 m/s; control group: 24.7 ± 2.1 m/s; the 30 μg PGE1-treated group: 24.4 ± 1.7 m/s; and the 100 μg PGE1-treated group: 24.9 ± 2.2 m/s; $P = 0.82$). MCV of normal rats progressively increased over time. In contrast, the MCV of untreated diabetic rats (control rats) was progressively decreasing. In the control group, the MCV at 2 and 4

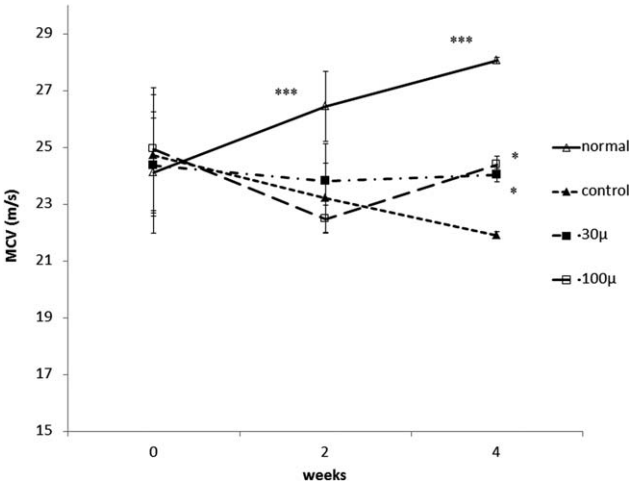

*Figure 4. Motor conduction velocity (MCV) at 0, 2, and 4 weeks after prostaglandin E1 (PGE1) administration. MCV was significantly lower in the control group when compared with the normal group at 2 and 4 weeks (P < 0.001). MCV was significantly higher in the PGE1-treated groups when compared with the control group at 4 weeks (P < 0.05). *P < 0.05 and ***P < 0.001 vs. control rats.*

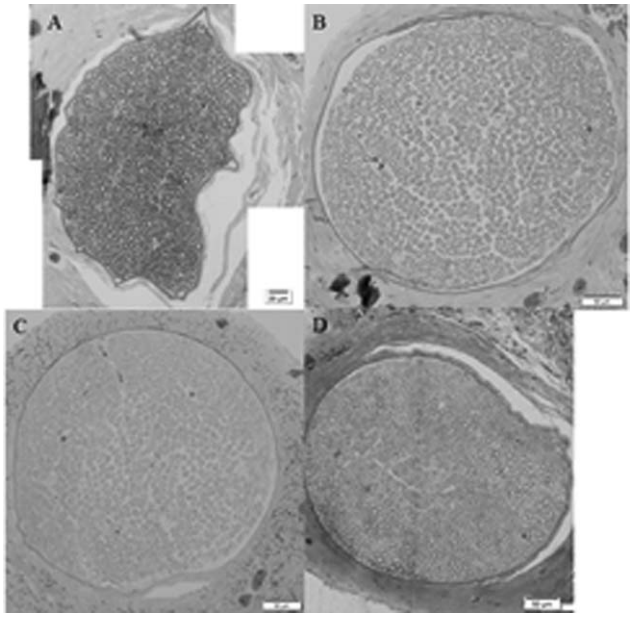

*Figure 5. Photomicroscopic views of myelinated fibers of the tibial nerve at 4 weeks: (**A**) normal group; (**B**) control group; (**C**) 30 μg PGE1 group; and (**D**) 100 μg PGE1 group. Intraneural edema was observed in diabetic groups. However, edema appears to be alleviated by the use of PGE1, especially in 100 μg group.*

weeks was 23.2 ± 1.2 m/s and 21.9 ± 0.1 m/s, respectively, and these were significantly lower than those of the normal group (2 weeks: 26.4 ± 0.5 m/s; 4 weeks: 28.1 ± 0.2 m/s; $P < 0.001$). In the 30 μg PGE1-treated group and the 100 μg PGE1-treated group, the MCV (30 μg group: 24.0 ± 0.2 m/s; 100 μg group: 24.4 ± 0.3 m/s) at 4 weeks were significantly higher than those of the

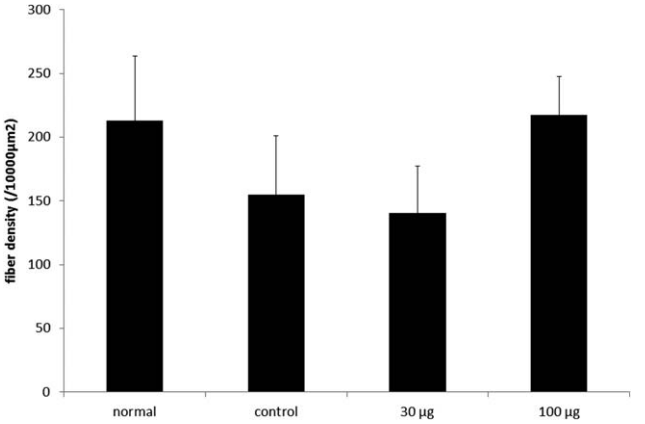

Figure 6. Histology of the tibial nerve at 4 weeks after prostaglandin E1 administration. Fiber density was not significantly different among the four groups (P = 0.46).

control group (21.9 ± 0.1 m/s; $P < 0.05$). The MCV at 4 weeks in the PGE1-treated groups (30 μg group: 24.0 ± 0.2 m/s; 100 μg group: 24.4 ± 0.3 m/s) were significantly lower than that of the normal group (28.1 ± 0.2 m/s; $P < 0.01$; Fig. 4).

Histological Examinations

The myelinated nerve fiber population in tibial nerves was evaluated, in particular fiber density and diameter (Fig. 5). Fiber density was not significantly different among the four groups (normal group: $213.2 \pm 50.6 \times 10^{-4}$ μm^2; control group: $154.9 \pm 46.1 \times 10^{-4}$ μm^2; the 30 μg PGE1-treated group: $140.5 \pm 37.1 \times 10^{-4}$ μm^2; and the 100 μg PGE1-treated group: $217.2 \pm 30.6 \times 10^{-4}$ μm^2; $P = 0.46$). However, in the 100 μg PGE1-treated group, fiber density tended to be higher than that of the control group, although the difference was not statistically significant ($P = 0.09$; Fig. 6). Mean fiber diameter was not significantly different among the four groups (normal group: 4.11 ± 0.56 μm; control group: 3.93 ± 0.44 μm; the 30 μg PGE1-treated group: 4.02 ± 0.41 μm; and the 100 μg PGE1-treated group: 4.04 ± 0.35 μm; $P = 0.15$). There were no significant differences between the control and PGE1-treated (30 and 100 μg) groups ($P = 0.62$).

mRNA Expression

After 4 weeks of PGE1 administration, the NGF mRNA expression of the tibial nerve was significantly higher in the control group (1.75 ± 0.37) when compared with the normal group (1.00 ± 0.37; $P < 0.001$). Administration of PGE1 (both 30 and 100 μg) significantly decreased NGF mRNA expression levels (the 30 μg PGE1-treated group: 1.11 ± 0.48, the 100 μg PGE1-treated group: 1.44 ± 0.69) in the tibial nerve when compared with the control group (1.75 ± 0.37; $P < 0.001$ and $P < 0.01$; Fig. 7). Meanwhile, NGF mRNA expression

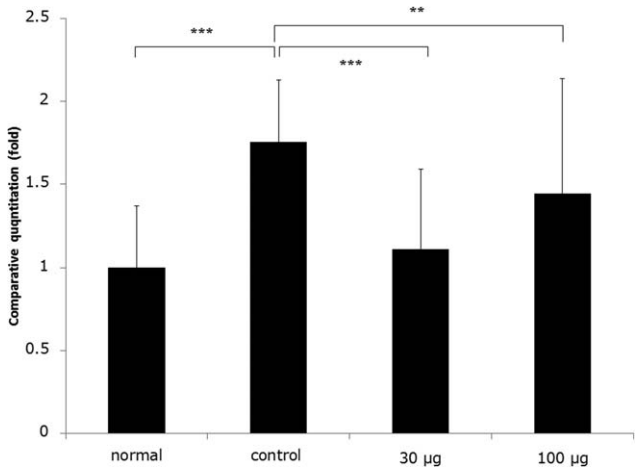

*Figure 7. Nerve growth factor (NGF) mRNA expression levels in the tibial nerve 4 weeks after prostaglandin E1 (PGE1) administration. NGF mRNA expression levels were significantly higher in the control group versus the normal group (P < 0.001). NGF mRNA expression levels were significantly lower in the PGE1-treated groups when compared with the control group (30 μg PGE1 group: P < 0.001; 100 μg PGE1 group: P < 0.01). **P < 0.01 and ***P < 0.001 vs. control rats.*

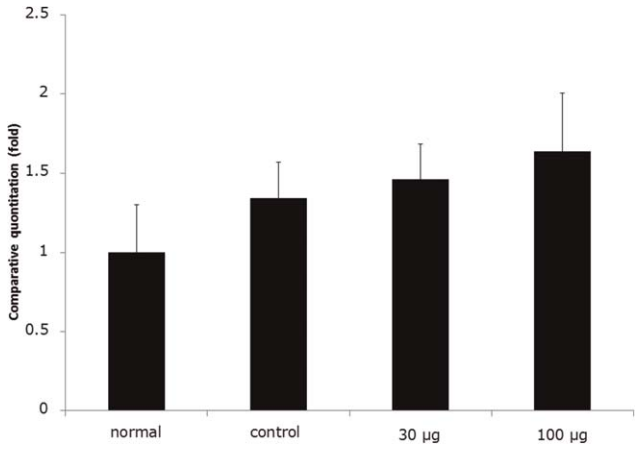

Figure 8. Nerve growth factor (NGF) mRNA expression levels in the dorsal root ganglia (DRG) 4 weeks after prostaglandin E1 administration. NGF mRNA expression levels in the DRG were not significantly different among the four groups (P = 0.34).

levels in the DRG were not significantly different among the four groups (control group: 1.34 ± 0.23; normal group: 1.00 ± 0.30; the 30 μg PGE1-treated group: 1.46 ± 0.23; and the 100 μg PGE1-treated group: 1.64 ± 0.37; $P = 0.34$; Fig. 8). In the control group, VEGF mRNA expression levels (0.89 ± 0.16) in the tibial nerve tended to be lower than that of the normal group (1.00 ± 0.21); however, it was not significantly different ($P = 0.78$). Administration of PGE1 (both 30 and 100 μg) significantly increased VEGF mRNA expression levels (the 30 μg PGE1-treated group: 1.91 ± 0.88; the

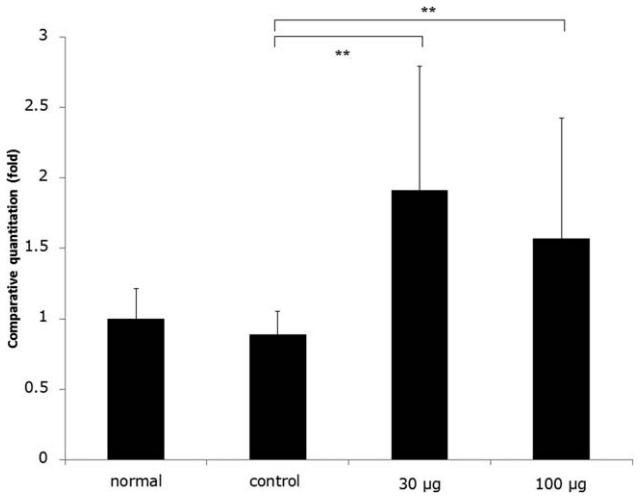

*Figure 9. Vascular endothelial growth factor (VEGF) mRNA expression levels in the tibial nerve 4 weeks after prostaglandin E1 (PGE1) administration. VEGF mRNA expression levels in the control group tended to be lower than the normal group; however, there was no significant differences (P = 0.78). VEGF mRNA expression levels were significantly higher in the PGE1-treated groups when compared with the control group (P < 0.01). **P < 0.01 vs. control rats.*

100 μg PGE1-treated group: 1.57 ± 0.85) in the tibial nerve when compared with the control group (0.89 ± 0.16; $P < 0.01$; Fig. 9).

DISCUSSION

Sustained hyperglycemia is harmful to the vascular tree and causes both macrovascular and microvascular dysfunction.[16] The precise nature of the injury to the peripheral nerves from hyperglycemia has yet to be clarified. However, the injury appears to be related to mechanisms such as polyol accumulation, injury from advanced glycation end products, and oxidative stress.[17,18] High glucose-induced inflammatory responses have been mechanistically linked to the production of reactive oxygen species and advanced glycation end products.[19]

Entrapment neuropathy associated with diabetes mellitus frequently involves the peroneal nerve at the level of the fibula head and the tibial nerve in the tarsal tunnel. Dellon[7] recommended tarsal tunnel release even for diabetics with poor plantar sensitivity and high risk for foot gangrene. According to Dellon, 80% of such patients demonstrate significant pain relief and more than only protective sensation. In addition, ulceration and subsequent amputation can be prevented in many of these patients. Dellon's approach has many supporters. However, not all supporters are fully satisfied with the effect of tarsal tunnel release. Caffee[8] performed tarsal tunnel release in patients with diabetes mellitus and could confirm restoration of sensation only in half of the cases.

Similarly, Wieman and Patel[9] reported poor results regarding its effects on foot sensitivity. Because Coffee[8] and Wieman and Patel[9] reported significant pain relief after surgery, Dellon's approach appears to provide some benefits to ailing diabetics. However, development of an adjuvant treatment to reinforce the effect of surgery on nerve recovery is highly desirable.

Zochdne et al. assessed sciatic–tibial conduction for more than 16 weeks in nondiabetic rats and rats rendered diabetic with STZ. Tibial mononeuropathy developed in both groups but appeared earlier in diabetic rats. Zochdne et al.[11] reported that rats reared on wire grid flooring develop electrophysiological abnormalities that are confined to sciatic–tibial motor fibers within the first 2 weeks of the experiment and that these abnormalities persist at 4 weeks. In the current study, we addressed the question of whether PGE1 alleviates entrapment neuropathy associated with hyperglycemia using the animal model proposed by Zochdne et al. and successfully demonstrated its beneficial effects using electrophysiological and behavioral tests.

Several observations may explain the mechanisms behind these effects. PGE1 has been widely used in microsurgical reconstructive procedures because of its long-lasting anti-ischemic and tissue-protecting effects against reperfusion injury.[20] Fang et al.[21] reported that PGE1 suppresses tumor necrosis factor-induced inflammation by inhibiting nuclear factor-κB activation and production of reactive oxygen species. Therefore, PGE1 may inhibit systemic inflammation in diabetic mellitus. Many reports have previously suggested that PGE1 improves electrophysiological function by increasing blood flow and metabolism in animal models of diabetic neuropathy. Aside from increasing oxygen delivery via its vasotropic effects,[22] PGE1 may have direct effects on neurons. For example, PGE1 induces the expression of c-Fos and Myc proteins in rat hippocampal cells and protects these cells from hypoxic injury.[23] PGE1 protects cultured spinal neurons from the effects of nitric oxide toxicity[24] and induces depolarization of sensory nerve terminals.[25] In addition, PGE1 prevents neuronal apoptosis, which is induced in the superficial dorsal horn of the rat spinal cord by sciatic nerve constriction.[26]

Regarding pain alleviation with PGE1, we specifically focused on the expression of NGF and VEGF. NGF was originally described as a neurotrophic factor that is necessary for the promotion and survival of nociceptors and the sympathetic nervous system during development.[27] In adults, NGF is critically involved in the development and maintenance of chronic pain.[28–30] A recent study demonstrated a significant increase in the density of cutaneous NGF and trkA-positive intraepidermal nerve fibers in diabetic rats.[31] In peripheral nerve injuries or disorders, NGF is taken up by Schwann cells and axons surrounding severed axons[32] and transported to the DRG, where transcription of a variety of pain-related genes is accelerated.[33,34] In the current study, we demonstrated that NGF mRNA was significantly increased in the tibial nerve and appeared to be associated with mechanical allodynia in the supplying area. We also found that expression of NGF mRNA in the tibial nerve was significantly suppressed by systemic administration of PGE1, which led to significant improvements in both mechanical allodynia and neural function.

VEGF acts not only as an enhancer of vascular growth but also as a neurotrophic factor in the peripheral nervous system.[35,36] Diabetes mellitus significantly impairs VEGF production.[37] In fact, the current study demonstrated that the levels of VEGF mRNA at the entrapment site were lower in the diabetes mellitus group than in the nondiabetes mellitus group, although the difference was not statistically significant. Previous studies confirmed that Schwann cells increase VEGF production in response to chronic nerve compression,[38] and this increase in VEGF production is most pronounced in compressed sections.[39,40] Therefore, hypoxic conditions at the entrapment site likely promote the expression of VEGF, which in turn inhibits ischemic injury and promotes neural repair. The findings of the current study clearly demonstrated that PGE1 administration significantly enhanced VEGF mRNA expression at the entrapment site, thereby corroborating observations from previous studies that reported that VEGF is upregulated by PGE1.[41] Although we were unable to determine the exact mechanisms responsible for the decrease in NGF and the increase in VEGF induced by PGE1 at the entrapment site, we believe that these results, taken together, offer hints for developing novel treatment strategies for pain control in patients with diabetic neuropathy.

In conclusion, we demonstrated that multiple types of neurological deterioration, such as mechanical allodynia, hyperalgesia, and functional and electrophysiological abnormalities, in rats with STZ-induced diabetes mellitus can be alleviated by systemic administration of PGE1. Furthermore, the patterns of expression of NGF and VEGF suggested that PGE1 alleviated damage to peripheral nerves resulting from focal compression at the entrapment site by inhibiting NGF and enhancing VEGF production.

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