ELSEVIER

European Journal of Pharmacology 376 (1999) 257–264

ejp

www.elsevier.nl/locate/ejphar

The sympathomimetic agent, 6-hydroxydopamine, accelerates cutaneous wound healing

Lincoln R. Kim [a], Bruce Pomeranz [a,b,*]

[a] *Department of Physiology, Faculty of Medicine, University of Toronto, Rm. 401, 25 Harbord St., Toronto, Canada M5S 3G5*
[b] *Department of Zoology, Faculty of Arts and Sciences, University of Toronto, Rm. 401, 25 Harbord St., Toronto, Canada M5S 3G5*

Received 7 April 1999; received in revised form 17 May 1999; accepted 21 May 1999

Abstract

Using pharmacological stimulation of sympathetic terminals, the present study examines the role of sympathetic post-ganglionic neurons in cutaneous wound healing. Effects of local stimulation of sympathetic post-ganglionic neurons with 0.2 mg/kg 6-hydroxydopamine were studied on the healing of full-thickness skin incisions in rats. Epidermal wound healing was measured by a novel non-invasive quantitative method based on the increasing electrical resistance of healing skin. Dermal healing was determined by measuring wound breaking strength using an Instron Universal Testing device. We report a 35% increase in the rate of epidermal wound healing ($P < 0.05$, $n_e = 21$, $n_c = 18$) and a 43% increase in dermal strength ($P < 0.05$, $n_e = 13$, $n_c = 10$) after 6-hydroxydopamine treatment. Thus, our results show that pharmacological stimulation of sympathetic post-ganglionic neurons markedly accelerates skin wound healing at both the epidermal and dermal levels. This is the first study to show that peripheral nerve stimulation and specifically sympathetic stimulation accelerates cutaneous wound healing. We discuss these results in relation to neurogenic inflammation.

Keywords: Sympathetic nervous system; 6-Hydroxydopamine; Wound healing; Breaking strength; Neurogenic inflammation; Skin

1. Introduction

The role of peripheral nerves in cutaneous wound healing is poorly understood. Recent evidence has shown that peripheral denervation delays cutaneous wound healing (Engin et al., 1996). It has also been shown that stimulation of sympathetic post-ganglionic neurons promotes neurogenic inflammation (Coderre et al., 1989; Green et al., 1993a). Since inflammation is a major step in wound healing (Levenson et al., 1965) and sympathetic post-ganglionic stimulation promotes neurogenic inflammation, we tested the hypothesis that cutaneous wound healing is accelerated by sympathetic post-ganglionic stimulation.

While numerous studies have looked at neurogenic inflammation in the skin (Jancso et al., 1968; Chahl, 1988), very few have studied the role of nerves in skin wound healing (Wexler et al., 1975; Kay and LeWinn, 1981; Kjartansson et al., 1987). However the few studies which showed that sensory and sympathetic nerves affect wound healing were all based on ischemic skin flaps in which blood supply is limited by a small pedicle connecting the flap. Hence skin flaps emphasize the vasodilatory aspects of neurogenic effects (Kjartansson et al., 1987). In contrast, our study examined the influence of sympathetic nerves on the more common form of wounds, full thickness skin incisions. Since our skin incision model does not focus on ischaemia, nerve stimulation in our experiments could be mediating not only vasodilation but other aspects of neurogenic inflammation such as plasma extravasation and degranulation of mast cells (Jancso, 1960; Jancso et al., 1968, 1977; Chahl, 1988).

Neurogenic inflammation was previously considered to be governed entirely by sensory neurons (Chahl, 1988). However the sympathetic nervous system has recently been shown to significantly contribute to this process. Thus electrical and chemical stimulation of sympathetic efferents have resulted in vasodilation and increased plasma extravasation in human and rat skin (Gozsy and Kato, 1966; Blumberg and Wallin, 1987; Lundberg et al., 1989; Donnerer et al., 1991). Chemical activation of sympathetic post-ganglionic neurons using low doses of 6-hydroxy-

* Corresponding author. Tel.: +1-416-978-8646; fax: +1-416-978-8532

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00391-X

dopamine was reported to produce a prolonged increase in plasma extravasation in skeletal joints (Coderre et al., 1989; Green et al., 1993a; Green et al., 1994). Also chemical sympathectomy was found to significantly attenuate plasma extravasation in rat skin (Helme and Andrews, 1985; Khalil and Helme, 1989; Green et al., 1993b). Consequently, since sympathetic post-ganglionic neurons have been shown to mediate inflammation and since inflammation is an important stage in healing, we hypothesize that sympathetic post-ganglionic stimulation may mediate wound healing.

We tested this hypothesis by stimulating the sympathetic post-ganglionic neurons with 6-hydroxydopamine, a highly specific sympathomimetic for post-ganglionic neurons. The effects of 6-hydroxydopamine are concentration dependent. High doses chemically denervate sympathetic post-ganglionic neurons while low doses stimulate these neurons (Thoenen and Tranzer, 1973; Kostrzewa and Jacobowitz, 1974). The present study employed low doses of 6-hydroxydopamine injected locally to stimulate the sympathetic post-ganglionic neurons during the inflammatory period (i.e., the first 4 days post-operatively) to determine its effects on cutaneous wound healing.

After making a skin incision, wound healing was monitored for fourteen days using two methods: transcutaneous electrical resistance for epidermal healing and wound breaking strength measurements for dermal healing. Hence this study examines the effect of chemical stimulation of sympathetic post-ganglionic neurons on epidermal and dermal healing of skin incisions.

2. Materials and methods

2.1. Studies to determine optimal dose of 6-hydroxydopamine

In order to find the optimal dose to stimulate the sympathetic post-ganglionic neurons, anaesthetized rats (with intact skin) were repeatedly injected subcutaneously with varying small doses of 6-hydroxydopamine in varying volumes of vehicle (1% ascorbic acid) in a blind design ($n = 9$ rats). The doses varied from 0.05 to 4 mg/kg and the volumes injected varied from 0.25 to 1 ml. Previous studies have shown that at low doses (< 10 mg/kg), 6-hydroxydopamine causes the release of the large and small dense core vesicles in the sympathetic post-ganglionic neurons terminals (Thoenen and Tranzer, 1973; Kostrzewa and Jacobowitz, 1974). The endpoint in our study was the ability of 6-hydroxydopamine to repeatedly produce localized piloerection, an indicator of sympathetic stimulation, in the vicinity of the injection (Thoenen and Tranzer, 1973). The dose and volume which produced the maximal piloerection with the longest duration was deemed optimal for the subsequent experiments. This was determined to be 0.2 mg/kg in 0.1 ml of vehicle.

Moreover, on five rats, 0.2 mg/kg (the optimal dose) was subcutaneously injected daily for 4 days to rule out the possibility that 6-hydroxydopamine had chemically denervated the sympathetic post-ganglionic neurons since high doses of 6-hydroxydopamine (> 50 mg/kg) are known to cause sympathectomy (Thoenen and Tranzer, 1973). The endpoint was the ability of 6-hydroxydopamine to produce localized touch domes (i.e., goose bumps), another indicator of sympathetic stimulation (Gloster and Diamond, 1992), in similar regions of the skin on days 0 and 4. The continued production of touch domes on day 4 would indicate that denervation had not occurred.

2.2. Sympathetic stimulation and wound healing

Male Wistar rats (Charles River), weighing 250–400 g, were housed under a controlled 12-h light cycle with food and water ad libitum. The rats were randomly assigned prior to the study into experimental ($n = 21$) and control ($n = 18$) groups. The experimental group (6-hydroxydopamine$_{s.c.}$) received subcutaneous injections of low-dose 6-hydroxydopamine (hydrobromide, Sigma, St. Louis, MO) to activate local sympathetic post-ganglionic neurons while the control group (vehicle$_{s.c.}$) received injections of the vehicle (1% ascorbic acid). At a dose of 0.2 mg/kg (determined to be the optimum dose from the piloerection studies described above), 0.1 ml of freshly prepared 6-hydroxydopamine in a vehicle of 1% ascorbic acid was injected subcutaneously 1 cm lateral to the centre of the wound on both sides. This was done on a lightly anaesthetized animal on days 0, 1, 2, 3 post-wounding. The injection of 6-hydroxydopamine was administered daily for the first 4 days post-wounding because it is within this time period that the inflammatory phase of wound healing is maximal. Control animals were similarly injected with the vehicle, 1% ascorbic acid.

2.3. Anaesthesia

Rats were anaesthetized prior to surgery, drug injection, and skin testing sessions with 1.5 ml/kg of a 1:7:1 cocktail i.p. comprised of Atravet (50 mg/ml acepromazine maleate), Rogarsetic (100 mg/ml ketamine hydrochloride) and Rompun (20 mg/ml xylazine hydrochloride), respectively, and remained anaesthetized for approximately 1 h. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.4. Surgery

Anaesthesized rats received a 1-cm full-thickness interscapular incision oriented rostro-caudally which penetrated the skin and subcutaneous fat down to the panniculus carnosus muscle. The gaping wounds were not sutured and hence were left to heal by secondary intention. The healing

was monitored for the next 14 days by two methods: transcutaneous electrical resistance and wound breaking strength measurements.

2.5. Transcutaneous electrical resistance

A novel method for measuring epidermal healing (Spence and Pomeranz, 1996) which monitors transcutaneous electrical resistance was used to quantify the progress of healing of this wound. This technique of wound healing measurement is based on the fact that intact skin is relatively impermeable to ions (Edelberg, 1967) and hence has a much higher electrical resistance than wounded skin (Spence and Pomeranz, 1996). With healing, the epithelium becomes increasingly impermeable to ions as the ionic barrier is re-established by the stratum corneum (Lykken, 1971; Spence and Pomeranz, 1996). As the wound heals and the ability to pass ionic current through the wound decreases, Ohm's Law predicts that the resistance increases proportionately. Skin incisions were made and the progress of wound healing was measured by transcutaneous electrical resistance on days 0, 4, 7, 11 and 14 post-wounding for each animal. Animals were shaved prior to surgery and on subsequent testing days. Details of the transcutaneous electrical resistance method have been published elsewhere (Spence and Pomeranz, 1996). We give a brief synopsis here as follows.

Transcutaneous electrical resistance was measured using Ag/AgCl nonpolarizing electrodes. The recording electrode (length (l) = 3.5 cm, diameter = 0.1 cm) consisted of a Ag/AgCl wire encased in a plastic tube (l = 3.5 cm, diameter = 0.5 cm) filled with 0.9% USP saline. Using a micromanipulator this electrode was gently applied topically in a perpendicular fashion touching the surface of the wounds without disrupting the healing process. This was applied for 2 min each time and was done repeatedly on days 0, 4, 7, 11 and 14. A cellulose nitrate millipore filter (Sartorius SM) covering the bottom of the tube (with 0.1-mm pores) provided a slow but steady supply of saline to the skin surface. The steady stream of saline permeated through scabs and scar tissue and allowed for stable resistance readings within 2 min of applying the recording electrode to the skin surface.

The reference electrode, a Ag/AgCl plate (l = 3.5 cm, width (w) = 1 cm, thickness = 0.1 cm), was inserted subcutaneously in the dorsal right hindlimb of the rat prior to each recording session. The considerable distance of the reference electrode from the wound site was chosen to ensure that the current would pass from the recording electrode through the wound, into the body core, and out to the reference electrode (i.e., not over the surface of the skin). Both the recording and reference electrodes were connected to a HIOKI 3234 Printing Multimeter (Hioki, Nagano, Japan) to record DC ohmic resistance.

Resistance measures were recorded every 30 s until stable readings were attained within 2 min. Simple precautions prevented polarization: (a) chloridized electrodes (b) large electrode surfaces and (c) adequate saline to interface the electrode and tissues. We tested for electrode polarization by periodically reversing the leads and never found a problem. In addition the recording and reference electrodes were short-circuited before every testing session to measure the resistance of the system (including the chloridized Ag electrodes) which was less than 2 kΩ. Preliminary results have shown that resistance measures taken on day 0 on a freshly cut wound are 0.3–1 kΩ higher than the short out resistance demonstrating that the resistance of the body core is negligible.

Healing rates were determined from the slopes of a semi-logarithmic graph of electrical resistance (kΩ) vs. time (days). Linear regression analysis (by Statsview 1.0) was used to determine the slope for each animal as exemplified in Fig. 1.

Testing was stopped on the day that the resistance surpassed 40 kΩ for the following reasons. (1) Histological studies in our lab show that all wound resistances > 40 kΩ correlated with an intact stratum corneum, which is the final result of epidermal wound healing (Spence and Pomeranz, 1996). Thus, a reading of 40 kΩ and one of 1000 kΩ would both represent healed wounds with little difference in their healing. (2) Intact skin, which has never been wounded, shows resistances of 40 kΩ or greater, so we concluded that the healing curve reached completion

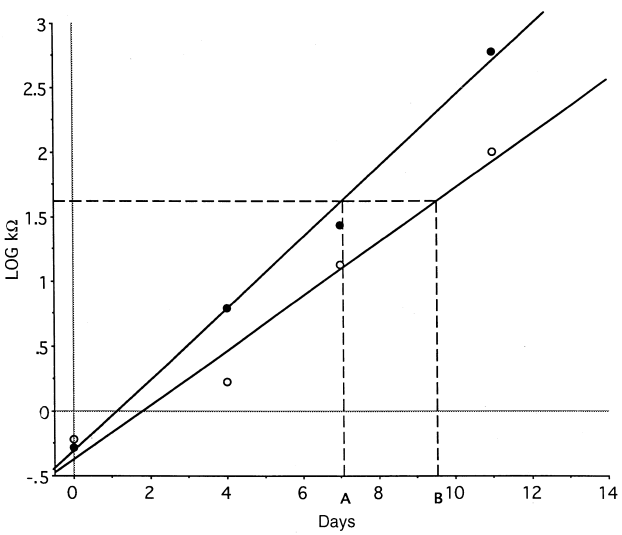

Fig. 1. Examples of resistance healing slopes. Healing rate as determined from the slope of the graph of resistance (kΩ) vs. time (days post-wounding). These show typical regression slopes of a 6-hydroxydopamine experimental rat (closed circles) and vehicle control rat (open circles). The slope of the experimental rat is 0.27 log kΩ/day and the slope of the control rat is 0.20 log kΩ/day. For this linear regression analysis (by Statsview 1.0), the resistance (in kΩ) on the y-axis were transformed to log 10 values. The regression coefficients for the experimental and control regression lines were 0.99 and 0.98, respectively. The dash lines indicate the number of days required to reach 40 kΩ resistance (1.6 on the log scale). The experimental rat reached 40 kΩ at 7.1 days (point A) while the control rat took 9.6 days (point B).

when 40 kΩ was surpassed. (3) Healed skin would cause plateauing of the healing graphs (see Fig. 1) due to a ceiling effect, which interferes with the linear regression analysis.

Transcutaneous electrical resistance has been validated in our laboratory using histology (Spence and Pomeranz, 1996), which shows that resistance rises during epidermal healing and that higher resistances were due to re-establishment of stratum corneum (formed by the keratin deposited by keratinocytes).

2.6. Wound breaking strength

In addition to repeated resistance measurements on each rat, wound breaking strength was measured once at the end of the healing period as this was a terminal experiment. Breaking strength is a well known method of evaluating the extent of the wound's ability to withstand tearing (Levenson et al., 1965). As opposed to tensile strength which incorporates the total cross-sectional area of the wound (good for homogenous industrial materials), breaking strength largely measures the strongest layer in a heterogenous material such as skin. This would represent the collagen organization in the dermal levels of the skin (Scott et al., 1985), and hence is a good measure of dermal healing.

Breaking strength was measured using an Instron Universal Testing Device (Universal 4200) on days 11 or 15 post-operatively (days 11 and 15 measurements were done on different animals as this was a terminal experiment). A 4.0×0.5 cm ($l \times w$) segment of skin cut perpendicular to and through the center of the wound was removed from euthanized animals. The Instron device applied a constant load under computer control at 10 mm/min until the sample tore along the line of the healed wound. The breaking strength was then recorded electronically from the Instron device in kg.

2.7. Visual evaluation

The wounds were evaluated qualitatively on days 0, 1, 2, 3, 4, 7, 11 and 14 to visually monitor the wound's healing. Photographs were also taken on each of the testing days using a Nikon Medical-Nikkor camera with a close-up lens of 2× to be assessed by blinded observers at the end of the experiment.

2.8. Control i.p. study

In 10 rats, a further control was done by giving 0.2 ml of 0.2 mg/kg 6-hydroxydopamine intraperitoneally (instead of subcutaneously) to rule out systemic effects of the low dose 6-hydroxydopamine treatment. This would determine if the effects of 6-hydroxydopamine$_{s.c.}$ were due to local stimulation of sympathetic post-ganglionic neurons. In this control group, transcutaneous electrical resistance was monitored for two weeks and breaking strength was measured on day 15 post-wounding.

2.9. Statistical analyses

The results are expressed as means ± standard error. An ANOVA (analysis of variance) followed by the Fisher's Test was used for the resistance studies as three groups were compared (6-hydroxydopamine$_{s.c}$, vehicle$_{s.c}$, and 6-hydroxydopamine$_{i.p.}$). Similarly, an ANOVA followed by the Fisher's Test was used for the breaking strength studies to compare five groups (6-hydroxydopamine $_{s.c,\ day\ 11}$, vehicle $_{s.c,\ day\ 11}$, 6-hydroxydopamine $_{s.c,\ day\ 15}$, vehicle $_{s.c,\ day\ 15}$, and 6-hydroxydopamine$_{i.p,\ day\ 15}$). Significance level was taken as 0.05.

3. Results

3.1. Studies to determine optimal dose of 6-hydroxydopamine

In the piloerection study designed to optimize the dose, a 0.2 mg/kg dose of 6-hydroxydopamine was determined to produce not only the maximal piloerection but also an effect with the longest duration (30–60 min). Moreover, it was observed that the touch domes encompassing the same area could be reproduced after 4 days of repeated injections with this dose. This indicates that the sympathetic post-ganglionic neurons were still intact and hence not damaged by previous 6-hydroxydopamine low dose injections on days 0, 1, 2 and 3. It should be noted that the dosage of 6-hydroxydopamine required to produce sympathectomy is > 50 mg/kg (Kostrzewa and Jacobowitz, 1974), which is over 250 times larger than the dose used in this study for sympathetic post-ganglionic neuronal stimulation.

3.2. Transcutaneous electrical resistance

Transcutaneous electrical resistance was measured on days 0, 4, 7, 11 and 14 post-wounding. Regression slopes were determined for the electrical resistances of each rat (as exemplified in Fig. 1) to determine the rate of healing. The mean rate of wound healing for the experimental rats (6-hydroxydopamine$_{s.c}$; $n = 21$) was significantly greater than the vehicle slopes (vehicle$_{s.c}$; $n = 18$) (0.27 ± 0.02 vs. 0.20 ± 0.02 log (kΩ)/day, respectively; $P < 0.05$). Mean regression coefficients were 0.98 for the experimentals and 0.96 for the controls indicating that the healing slopes were reliable. These healing rates are summarized in Fig. 2. The results show a 35% acceleration in the rate of healing at the epidermal level. In addition, the time for the experimental and control wounds to reach 40 kΩ (Fig. 3) was significantly decreased (8.15 ± 0.44 vs. 10.68 ± 0.69 days, respectively; $P < 0.05$), resulting in a 2.5-day

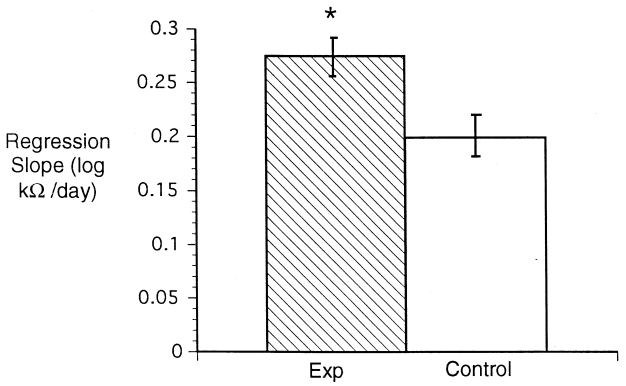

Fig. 2. Sympathetic post-ganglionic stimulation accelerates healing. Comparison of mean regression slopes (rates of healing) between low dose 6-hydroxydopamine (exp, cross-hatched bar) and vehicle (control, open bar) treated rats. The mean regression slopes of the experimental ($n = 21$) and control ($n = 18$) groups are 0.27 log kΩ/day $\pm$ 0.02 (S.E.M.) and 0.20 log kΩ/day $\pm$ 0.02 (S.E.M.), respectively. The asterisk indicates that these slopes are significantly different ($P < 0.05$). The mean regression coefficients for experimental and control slopes are 0.98 and 0.96, respectively.

acceleration in wound healing. Hence low dose 6-hydroxydopamine produced marked acceleration of healing at the epidermal level.

3.3. Wound breaking strength

Wound breaking strength was measured on days 11 and 15 post-wounding. It was determined to be significantly greater for the experimental rats (6-hydroxydopamine$_{s.c}$, $n = 13$) than the vehicle-treated rats (vehicle$_{s.c}$, $n = 10$) on day 15 (0.67 ± 0.07 vs. 0.47 ± 0.05 kg, respectively; $P < 0.05$) but not significantly different on day 11 (0.44 ± 0.04 vs. 0.39 ± 04 kg, respectively; $P < 0.05$, $n = 7$ for the experimental group and $n = 7$ for the control group). This is summarized with histograms in Fig. 4. The results on day 15 represent a 43% increase in breaking strength produced by low dose 6-hydroxydopamine over control values. These results show that 6-hydroxydopamine stimulation of sympathetic terminals accelerates healing at the dermal level by day 15.

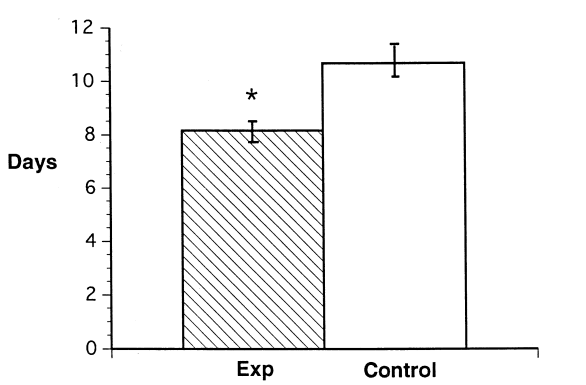

Fig. 3. Sympathetic post-ganglionic stimulation decreases healing time. Comparison of mean days to reach 40 kΩ between low dose 6-hydroxydopamine (exp, cross-hatched bar) and control (control, open bar) treated rats. The mean days to reach 40 kΩ of the experimental ($n = 21$) and control ($n = 18$) groups were 8.15 days $\pm$ 0.44 (S.E.M.) and 10.68 days $\pm$ 0.69 (S.E.M.), respectively. The asterisk indicates that these values were significantly different ($P < 0.05$). This shows that the experimental group reached 40 kΩ 2.5 days faster than the control group.

3.4. Visual evaluation

It was quite obvious by visual examination that the experimental wounds had healed faster than the control wounds based on the rate of disappearance of the fibrin clot and the healed appearance of scar tissue. By day 11, all 21 experimental wounds had lost the fibrin clot and had become scar tissue; whereas, eight of the 18 control wounds still had evidence of a fibrin clot. As these were subjective observations, no statistics were analyzed.

3.5. Control i.p. studies

Another separate study was done to determine whether the low-dose 6-hydroxydopamine was working via a localized mechanism involving the sympathetic terminals (i.e., was not a systemic or ganglionic effect). The 0.2 mg/kg 6-hydroxydopamine dose injected subcutaneously (6-hydroxydopamine$_{s.c}$; $n = 21$) locally near the wound had significantly greater wound healing rates and breaking

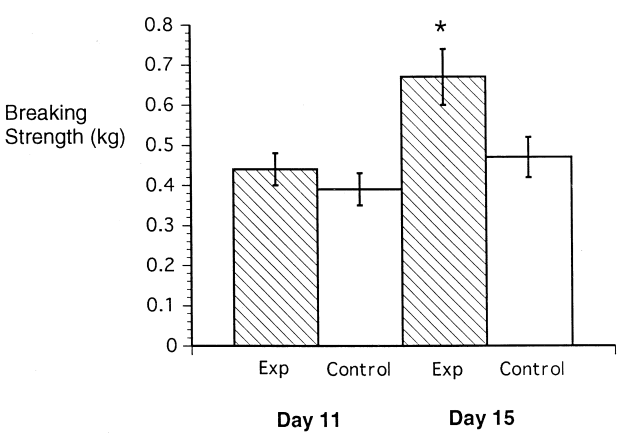

Fig. 4. Sympathetic post-ganglionic stimulation increases breaking strength. Comparison of mean breaking strengths between low dose 6-hydroxydopamine (exp, cross-hatched bar) and vehicle (control, open bar) treated rats on days 11 and 15 post-surgery. The mean breaking strengths of the experimental and control groups on day 11 were 0.44 kg $\pm$ 0.04 (S.E.M.) and 0.39 kg $\pm$ 0.04 (S.E.M.), respectively. Day 15 mean breaking strengths for experimentals and controls were 0.67 kg $\pm$ 0.07 (S.E.M.) and 0.47 kg $\pm$ 0.05 (S.E.M.). As indicated by the asterisk, day 15 mean breaking strengths between experimentals and controls was significantly different ($P < 0.05$) but day 11 results were not significantly different ($P < 0.05$). The experimental and control groups consisted of 13 and 10 rats, respectively, on day 15. There were seven rats in each group on day 11. Different rats were used in day 11 and day 15 studies as these were terminal experiments.

strengths than when it was injected intraperitoneally (6-hydroxydopamine$_{i.p}$; $n = 10$) during the same 4-day period on a separate group of rats ($P < 0.05$, ANOVA). The mean values were as follows: healing rates were 0.27 ± 0.02 log(kΩ)/day for the s.c. rats vs. 0.21 ± 0.01 log (kΩ)/day for the i.p. rats and day 15 breaking strengths were 0.67 ± 0.07 kg for the s.c. rats vs. 0.53 ± 0.03 kg for the i.p. rats. In addition, compared to vehicle s.c.-treated rats, the i.p. administration had no significant effect on wound healing at the epidermal and dermal levels (vehicle$_{s.c}$; $n = 18$). The i.p.-treated group showed the same wound healing slopes as the control rats ($P < 0.05$). Similarly the mean day 15 wound breaking strength of the i.p.-treated rats was not significantly different from vehicle s.c.-treated rats ($P < 0.05$). These results rule out the possibility that the low dose 6-hydroxydopamine had systemic effects and is consistent with its well known local stimulatory effects on sympathetic terminals (Kostrzewa and Jacobowitz, 1974). The mean regression coefficient for the 6-hydroxydopamine$_{i.p.}$ animals was 0.98.

4. Discussion

This study demonstrates that the local activation of sympathetic post-ganglionic neurons accelerates the rates of cutaneous wound healing at both the epidermal and dermal levels. First, we found a 35% increase in the rate of cutaneous wound healing at the epidermal level for the sympathetically-activated animals. This translates to an approximately 2.5-day difference between the rates of wound healing between experimental and control animals. Second, we found that the extent of dermal healing for the experimental group was significantly greater than that of the control group: on day 15 post-wounding there was a 43% increase in the wound breaking strength of the sympathetically-activated animals as compared to controls. Thus, locally injected low dose 6-hydroxydopamine accelerates skin wound healing on both the epidermal and dermal levels. The fact that intraperitoneal injections did not affect wound healing ruled out systemic effects suggesting the mobilization of solely local mechanisms in the subcutaneous injection experiments.

As stated in Section 1, the effects of 6-hydroxydopamine are concentration dependent: high doses cause chemical sympathectomy (Thoenen and Tranzer, 1973; Kostrzewa and Jacobowitz, 1974), whereas low doses are sympathomimetic. In an another study by our group, where high doses of 6-hydroxydopamine were used to cause sympathetic denervation, cutaneous wound healing was attenuated, indicating that the sympathetic post-ganglionic neurons are necessary for normal cutaneous wound healing (Kim et al., 1998). Taken together, the results of our two studies (acceleration of healing by 6-hydroxydopamine stimulation and inhibition of healing by 6-hydroxydopamine sympathectomy) are the first to suggest that sympathetic post-ganglionic neurons play a role in the regulation of wound healing.

The failure to see dermal effects on day 11 is consistent with past studies which showed that there is a net loss of collagen from 7 to 14 days due to rapid remodeling (Scott et al., 1985). Hence we only saw effects on day 15 and not on day 11 but this requires further studies on later days.

We did not use histology in this study and preferred transcutaneous electrical resistance for several reasons. First, in a previous study the transcutaneous electrical resistance method was validated by histology (Spence and Pomeranz, 1996). Second, problems with histology arose from difficulties encountered in fixation and cutting of the stratum corneum which was frequently destroyed in the processing of skin. Hence differences between experimental and control groups were difficult to show because of these serious artefacts. In contrast, the transcutaneous electrical resistance method was used in vivo where these artefacts were not encountered. And third, transcutaneous electrical resistance was a more objective quantitative method than histology.

In addition, transcutaneous electrical resistance has several advantages. It is non-invasive, reliable, and direct. Moreover, it gives a dynamic picture of healing allowing for repeated measurements over time in a given animal.

4.1. Neurogenic inflammation

Since sympathetic post-ganglionic stimulation promotes neurogenic inflammation and inflammation is important for wound healing, we propose that sympathetic post-ganglionic stimulation may accelerate cutaneous wound healing by increased neurogenic inflammation.

There is an apparent paradox in sympathetic neurogenic inflammation because the principal sympathetic neurotransmitter, noradrenaline, is usually thought to be a vasoconstrictor which should inhibit inflammation. However, the sympathetic terminals in the skin also contain proinflammatory peptides and purines which may promote inflammation by non-vasoconstrictory mechanisms (Gozsy and Kato, 1966; Coderre et al., 1989; Green et al., 1993a). In addition to noradrenaline, the other principle neurotransmitters present in the sympathetic terminals in the skin are neuropeptide Y, adenosine A2, and adenosine triphosphate (Schotzinger and Landis, 1990). Although neuropeptide Y has been shown to be a vasoconstrictor (Lundberg et al., 1982; Gibbons, 1992), Adenosine triphosphate and A2 have been shown to promote vasodilation, as well as, plasma extravasation (Chahl, 1977; Green et al., 1991; Robson and Heggers, 1992). More interestingly, activation of sympathetic post-ganglionic neurons causes the de novo synthesis of prostaglandin E2 a potent pro-inflammatory mediator (Cooper and Malik, 1985; Malik, 1988; Gonzales et al., 1991; Sherbourne et al., 1992), as well as, a

stimulator of collagen synthesis and epidermal keratinization (Robson and Heggers, 1992).

5. Conclusion

In conclusion, the present results demonstrate that low dose 6-hydroxydopamine, when locally injected subcutaneously in rat skin, markedly enhances cutaneous wound healing of full-thickness incisions at the epidermal and dermal levels. Sympathetic post-ganglionic stimulation resulted in acceleration of epidermal and dermal healing. Epidermal healing was 35% faster and dermal healing was 43% faster than control rats. This is the first paper to show a beneficial effect of peripheral nerve stimulation in incisional wound healing, and more specifically, the acceleration of healing by sympathetic stimulation.

Acknowledgements

This work was supported by the National Science and Engineering Research Council (NSERC) of Canada.

References

Blumberg, H., Wallin, B.G., 1987. Direct evidence of neurally mediated vasoconstriction in hairy skin of the human foot. J. Physiol. 382, 105–121.

Chahl, L.A., 1977. Interactions of substance P with putative mediators of inflammation and ATP. Eur. J. Pharmacol. 44, 45–49.

Chahl, L.A., 1988. Antidromic vasodilatation and neurogenic inflammation. Pharmacol. Ther. 37, 275–300.

Coderre, T.J., Basbaum, A.I., Levine, J.D., 1989. Neural control of vascular permeability, Interactions between primary afferents mast cells and sympathetic efferents. J. Neurophysiol. 62, 48–58.

Cooper, C.L., Malik, K.U., 1985. Prostaglandin synthesis and renal vasoconstriction elicited by adrenergic stimuli are linked to activation of alpha-1 adrenergic receptors in the isolated rat kidney. J. Pharmacol. Exp. Ther. 233, 24–31.

Donnerer, J., Amann, R., Lembeck, F., 1991. Neurogenic and non-neurogenic inflammation in the rat paw following chemical sympathectomy. Neuroscience 45, 761–765.

Edelberg, R., 1967. Electrical properties of skin. In: Brown, C.C. (Ed.), Methods in Psychophysiology. Williams and Wilkins, Baltimore, MD, pp. 1–53.

Engin, C., Demirkan, F., Ayhan, S., Atabay, K., Baran, N.K., 1996. Delayed effect of denervation on wound contraction in rat skin. Plast. Reconstr. Surg. 98 (6), 1063–1067.

Gibbons, I.L., 1992. Vasoconstrictor vasodilator and pilomotor pathways in sympathetic ganglia of guinea-pigs. Neuroscience 47, 657–672.

Gloster, A., Diamond, J., 1992. Sympathetic nerves in adult rats regenerate normally and restore pilomotor function during an anti-NGF treatment that prevents their collateral sprouting. J. Comp. Neurol. 326, 363–374.

Gonzales, R., Sherbourne, C.D., Goldyne, M.E., Levine, J.D., 1991. Noradrenaline-induced prostaglandin production by sympathetic postganglionic neurons is mediated by α2-adrenergic receptors. J. Neurochem. 57, 1145–1150.

Gozsy, B., Kato, L., 1966. Role of norepinephrine and 5-hydroxytryptamine in the delayed phase of the inflammatory reaction in rats. Int. Arch. Allergy Appl. Immunol. 30, 553–560.

Green, P.G., Basbaum, A.I., Helms, C., Levine, J.D., 1991. Purinergic regulation of bradykinin-induced plasma extravasation and adjuvant-induced arthritis in the rat. Proc. Natl. Acad. Sci. U.S.A. 88, 4162–4165.

Green, P.G., Luo, J., Heller, P.H., Levine, J.D., 1993a. Further substantiation of a significant role for the sympathetic nervous system in inflammation. Neuroscience 55, 1037–1043.

Green, P.G., Luo, J., Heller, P.H., Levine, J.D., 1993b. Neurogenic and non-neurogenic mechanisms of plasma extravasation in the rat. Neuroscience 52, 735–743.

Green, P.G., Luo, J., Heller, P.H., Levine, J.D., 1994. Effect of E-type prostaglandins on bradykinin-induced plasma extravasation in the knee joint of the rat. Eur. J. Pharmacol. 252, 127–132.

Helme, R.D., Andrews, P.V., 1985. The effect of nerve lesions on the inflammatory response to injury. J. Neurosci. Res. 13, 453–459.

Jancso, N., 1960. Role of the nerve terminals in the mechanism of inflammatory reactions. Bull. Millard Fillmore Hosp. 7, 53–77.

Jancso, N., Jancso-Gabor, A., Szolcsanyi, J., 1968. The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. Br. J. Pharmacol. 32, 32–41.

Jancso, G., Kiraly, E., Jancso-Gabor, A., 1977. Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270, 741–743.

Kay, S.R., LeWinn, L.R., 1981. Neural influences on experimental flap survival. Plast. Reconstr. Surg. 67, 42–48.

Khalil, Z., Helme, R.D., 1989. Sympathetic neurons modulate plasma extravasation in the rat through a non-adrenergic mechanism. Clin. Exp. Neurol. 26, 45–50.

Kim, L.R., Whelpdale, K., Zurowski, M., Pomeranz, B.H., 1998. Sympathetic denervation impairs epidermal healing in cutaneous wounds. Wound Repair Regener. 6 (3), 194–201.

Kjartansson, J., Dalsgaard, C.-J., Jonsson, C.-E., 1987. Decreased survival of experimental critical flaps in rats after sensory denervation with capsaicin. Plast. Reconstr. Surg. 79, 218–220.

Kostrzewa, R.M., Jacobowitz, D.M., 1974. Pharmacological actions of 6-hydroxydopamine. Pharmacol. Rev. 26, 199–288.

Levenson, S.M., Geever, E.F., Crowley, L.V., Oates, J.F., Berard, C.W., Rosen, H., 1965. The healing of rat skin wounds. Ann. Surg. 161, 293–308.

Lundberg, J.M., Terenius, L., Hökfelt, T., Martling, C.R., Tatemoto, K., Mutt, V., Polak, J., Bloom, S., Goldstein, M., 1982. Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta. Physiol. Scand. 116, 477–480.

Lundberg, J., Norgren, L., Ribbe, E., Rosen, I., Steen, S., Thorne, J., Wallin, B.G., 1989. Direct evidence of active sympathetic vasodilatation in the skin of the human foot. J. Physiol. 417, 437–446.

Lykken, D.T., 1971. Square-wave analysis of skin impedance. Psychophysiology 7, 262–275.

Malik, K.U., 1988. Interaction of arachidonic acid metabolites and the adrenergic nervous system. J. Med. Sci. 295, 280–286.

Robson, M.C., Heggers, J.P., 1992. Eicosanoids cytokines and free radicals. In: Cohen, K., Diegelmann, R.F., Lindblad, W.J. (Eds.), Wound Healing, Biochemical and Clinical Aspects, W.B. Saunders, Philadelphia, PA, pp. 292–315.

Schotzinger, R.J., Landis, S.C., 1990. Postnatal development of autonomic and sensory innervation of thoracic hairy skin in the rat. Cell Tissue Res. 260, 575–587.

Scott, P.G., Chambers, M., Johnson, B.W., Williams, H.T., 1985. Experimental wound healing, increased breaking strength and collagen synthetic activity in abdominal fascial wounds healing with secondary closure of the skin. Br. J. Surg. 72, 777–779.

Sherbourne, C.D., Gonzales, R., Goldyne, M.E., Levine, J.D., 1992. Norepinephrine-induced increase in sympathetic neuron-derived

prostaglandins is independent of neuronal release mechanisms. Neurosci. Lett. 139, 188–190.

Spence, D.W., Pomeranz, B., 1996. Surgical wound healing monitored repeatedly in vivo using electrical resistance of epidermis. Physiol. Meas. 17, 57–69.

Thoenen, H., Tranzer, J.P., 1973. The pharmacology of 6-hydroxydopamine. Annu. Rev. Pharmacol. 13, 169–180.

Wexler, M.R., Kalisman, M., Yeschua, R., Neuman, Z., 1975. The effect of phenoxybenzamine phentolamine and 6-hydroxydopamine on skin flap survival in rats. J. Surg. Res. 19, 83–87.