Effects of Chronic Sympathetic Stimulation on Corneal Wound Healing

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To explore the possibility that adrenergic fibers influence epithelial wound healing in the rabbit cornea, chronic electrodes were implanted in the left cervical sympathetic nerve, and symmetrical wounds were produced on the corneal epithelium of both eyes by means of n-heptanol. Continuous sympathetic stimulation was applied to awake, unrestrained animals during the wound-healing process (2–3 days) by use of a portable stimulator that delivered 1 ms, 3-Hz electric pulses at an amplitude necessary to evoke mydriasis. Migration rates of epithelial cells surrounding the wound and estimated wound closure times were calculated by measuring the reduction in wound size. In stimulated corneas, the epithelial migration rate was smaller (36.9 ± 6.2 μm/hr) than in control corneas (49.4 ± 5.4 μm/hr), the differences being significant (P < 0.05). Significant differences in wound closure times between stimulated (53.2 ± 6.8 hr) and control (41.3 ± 5.5 hr) corneas were also observed. These results suggest a modulatory influence of corneal adrenergic fibers on the processes that follow epithelial injury of the cornea. Invest Ophthalmol Vis Sci 28:221–224, 1987

The cornea receives a small number of adrenergic fibers from the superior cervical ganglion that run in the stroma and reach the epithelial layer.1–3 However, the role played by this sympathetic innervation has not been fully elucidated. It has been linked to the regulation of epithelial ion transport,4,5 as well as to the modulation of mitotic activity of the epithelial cells during wound healing.6 Friedenwald and Buschke7 reported a marked reduction of mitotic activity in the rat’s corneal epithelium following superior cervical ganglionectomy, an observation later confirmed in the rabbit.8 In addition, after removal of the superior cervical ganglion, noradrenaline released from degenerating nerve terminals decreased the mitotic activity of the epithelium, and this effect appeared to be mediated through an increase in cyclic-AMP; it was blocked by previous administration of propanolol.6,9

These sympathetic effects might be linked to the healing processes following corneal epithelial damage. Wounding the corneal surface is accompanied by stimulation of its pain sensory receptors,10 and intense nociceptive stimulation reflexly elicits autonomic responses, of which sympathetic activation plays a major role.11 Thus, it is possible that corneal adrenergic fibers are excited reflexly to contribute to wound healing. If this were the case, epithelial wound healing of the cornea should be affected by the amount of adrenergic activity reaching the eye. The purpose of this work has been to test this hypothesis by using chronic stimulation of the cervical sympathetic nerve to maintain a sustained activity of ocular adrenergic fibers and by evaluating the effects of stimulation on the healing of experimental corneal wounds. Preliminary results have been previously reported.12

Materials and Methods

Successful experiments were performed in eight albino rabbits of both sexes weighing 2.5–3.5 kg, unilaterally implanted with stimulating electrodes on the cervical sympathetic nerve. These preparations consistently responded to sympathetic stimulation. Animals were treated according to the ARVO Resolution on the Use of Animals in Research.

Electrode Implantation

The technique for chronic implantation of electrodes in the cervical sympathetic trunk has been described elsewhere.13 In brief, rabbits were anesthetized with sodium pentobarbitone (nembutal 30 mg/kg) slowly administered into the ear’s marginal vein. The preganglionic cervical sympathetic trunk was carefully dissected in the neck, and two wrapping electrodes (separated by a distance of 0.5–1 cm) were placed on the nerve. The electrodes were attached with sutures...
to neighboring muscles, and their lead wires exteriorized through a hole in the back of the neck. The success of the procedure was ascertained by the occurrence of mydriasis in response to electrical stimulation of the nerve after surgery and during subsequent days.

Corneal Wounding

After topical anesthesia with 2% propacaine, a corneal wound was produced in the epithelia of both eyes by applying for 30 sec a 9-mm², square-shaped piece of Whatman 1 paper embedded in n-heptanol in the center of the cornea. The eye was repeatedly washed afterwards with isotonic saline. The dimensions of the wound, stained with 2% fluoresceine, were measured every 4–6 hr with the calibrated eyepiece of a dissecting microscope (8X magnification) until complete healing was observed.

Sympathetic Stimulation

Unilateral electrical stimulation of the cervical sympathetic was delivered immediately after wounding the corneal epithelium. A small portable stimulator (FT Elektrotek, Kearns, UT) connected to the stimulating electrodes and secured in a bag on the back of the rabbit was employed for this purpose. It delivered a continuous train of 1 ms at 3-Hz pulses. Their intensity (usually 8–10 V) was adjusted to evoke a clear mydriatic response. The animals remained free in their cages during the intervals between measurements.

Analysis of Data

Corneal wound areas were determined from the lateral dimensions of the wound, and the radius of circles with equivalent areas were then calculated. The method developed by Crosson et al was used to analyze the decrease in equivalent wound radius. Migration rates were determined by linear regressions of the decrease in equivalent wound radius during the healing phase (8–30 hr) and were given by the slope of the regression line, being expressed as μm/hour. The time required for total closure of the corneal wound was calculated by extrapolation of the “best fit” of regression lines during the healing phase (8–30 hr) to 100% closure for each eye tested. Migration rates and the estimated time for wound closure in the stimulated and control eyes were compared using paired t-tests. Average values were expressed as mean ± SE. The level of significance for the differences was set at P < 0.05.

Results

Although the same procedure was used in all corneas to produce wounds of comparable size, a variation of 1–2 mm in the dimensions of their borders was observed 5 min after applications of n-heptanol; the mean initial surface of all wounds was 16.3 mm² ± 5.0 (n = 15). One of the corneas was excluded because sliding of the paper soaked with n-heptanol resulted in a large epithelial wound.

Figure 1 represents the mean reduction in wound surface (expressed as the percentage of the initial wound area) along the complete healing period in the control and the stimulated corneas. During the initial 10 hr, the evolution of wound closure was highly variable among individual corneas; moreover, there were no significant differences in wound surface reduction between control and stimulated eyes (61.8 ± 4.7% of the initial area in the control and 60.0 ± 3.4% in the stim-
ulated corneas). In fact, during the 5 hr following wound production, wound dimensions expressed as the percentage of the initial area increased slightly (10% or less) in four corneas, decreased less than 20% in another five, and reached a 20% reduction in the remaining seven. After about 10 hr, the healing rate of stimulated corneas became slower than in control corneas. The decrease in wound radius was used to quantify the healing phase because it is a linear process that is not affected by the initial wound size. The slopes of the regression lines of the wound radius of control and stimulated corneas represented the migration rates of epithelial cells surrounding the wound. These values were significantly smaller in the stimulated (36.9 ± 6.2 μm/hr) than in the control (49.4 ± 5.4 μm/hr) sides. This resulted also in significantly (P < 0.05) different estimated wound closure times, which were 41.3 ± 5.5 hr for the control and 53.2 ± 6.8 hr for the stimulated corneas. Figure 2 depicts the differences between rates of epithelial migration (left columns) and estimated rates of healing (right columns) in the control (white bars) and stimulated corneas (dashed bars).

Discussion

Corneal wounds heal initially as a result of epithelial cell migration from surrounding intact tissue that covers the denuded areas. The time course of wound closure observed in our control corneas is analogous to that described by other authors also using n-heptanol to induce epithelial wounding.14,16

The variability in the initial size of corneal wounds and in the occurrence of a latent phase, consistently present when epithelial damage was produced by mechanical means,15 might be explained by disruption of the cellular junctional complexes due to diffusion of n-heptanol to surrounding cells. This disruption of junctional complexes would allow fluorescein to stain the areas where epithelial cells remained attached to the basal lamina and would result in the appearance of a much larger wound than was actually produced. As n-heptanol is washed away by tears, the junctional complexes will likely reappear much faster than epithelial cells migrate, leading to a large decrease in wound size during the first 5 hr after wounding; however, when excessive diffusion of n-heptanol occurs, the surrounding cells may be lost during this period, and the wound size would possibly increase rather than decrease, as occurred with four corneas in the present work. Hence, changes of the latent phase consecutive to sympathetic stimulation could not be accurately evaluated in heptanol-induced corneal wounds.

A significant reduction in the rate of epithelial migration and in closure time of the corneal wound resulted from maintained adrenergic stimulation. These effects may be the consequence of direct sympathetic action on the epithelial cells or the indirect result of adrenergic influences on lacrimation and blinking. Stimulation of the preganglionic superior cervical ganglion or administration of adrenergic drugs vary the fluid flux and composition of lacrimal flow,17,18 whereas blinking may be affected by retraction of the nictitating membrane and the smooth muscle contraction.19 Either of these actions alters corneal humidification and can consequently influence the wound healing rate.

Continuous sympathetic stimulation at 3 Hz produces consistent pupillary and vascular effects in the rabbit.13 Under these conditions, each electrical pulse synchronously activates all the ganglion cells of the superior cervical ganglion at a frequency that is almost twice the spontaneous resting value found in sympathetic preganglionic fibers;20 thus, stimulation may induce the release of fairly large amounts of the endogenous neurotransmitter from all adrenergic fibers to the eye, not only in the cornea but in other ocular structures innervated by sympathetic nerves. This raises the question as to whether noradrenaline released by densely innervated structures like the iris could reach the corneal epithelium to retard corneal wound healing. Continuous sympathetic stimulation increases the aqueous humor levels of dopamine-beta-hydroxylase, the enzyme that accompanies noradrenaline release from adrenergic nerve terminals.21 However, the basal levels of noradrenaline found in the aqueous humor of animals and man22 are very low, suggesting that there...
exists in the aqueous a highly effective neutralizing mechanism for this neurotransmitter. Thus, it seems reasonable to suggest that the retarding effects of sympathetic stimulation on corneal wound healing are due to a direct effect of corneal adrenergic fibers on epithelial cells.

Beuerman et al. have recently reported that superior cervical ganglionectomy accelerates the migration rate of epithelial cells during corneal wound healing. Our results extend this observation and further support the hypothesis that corneal adrenergic fibers influence the healing processes after corneal wounding. The functional role of a slowing of epithelial sliding when the sympathetic system is activated is not obvious, but it may be linked to the modulation of the mitotic rate induced on these cells by the adrenergic agents.

Key words: cornea, wound healing, sympathetic stimulation, adrenergic system

Acknowledgment

The authors wish to thank Dr. Craig E. Crosson for his comments and assistance in analysis of data presented in this manuscript.

References