Naltrexone, an Opioid Antagonist, Facilitates Reepithelialization of the Cornea in Diabetic Rat

Ian S. Zagon,[1] Joe B. Jenkins,[2] Joseph W. Sassani,[3] James D. Wylie,[1] Torre B. Ruth,[1] Jamie L. Fry,[1] C. Max Lang,[2] and Patricia J. McLaughlin[1]

Ulcers and erosions of the corneal epithelium, as well as delays in resurfacing of the cornea after wounding, are major causes of ocular morbidity and visual loss in diabetes. To study whether intervention by the opioid antagonist naltrexone (NTX; 30 mg/kg, twice daily) can restore reepithelialization in diabetic cornea, we induced diabetes in rats by intravenous injection of 65 mg/kg streptozotocin. After confirmation of diabetes, 5-mm-diameter epithelial defects that did not include the limbus were created by mechanical scraping of the cornea. At 4 and 8 weeks, corneal reepithelialization was markedly subnormal, with delays ranging from 11% to 17-fold in the diabetic animals compared with control counterparts. Rats that were diabetic for 8 weeks also had a significant decrease in the incidence of complete wound closure. At 4 and 8 weeks, diabetic animals that were receiving NTX had an acceleration in reepithelialization compared with diabetic animals that were receiving vehicle and even surpassed controls. DNA synthesis in the corneal epithelium of diabetic rats was decreased up to 90% of control levels, and NTX exposure of diabetic subjects elevated the labeling index by up to eightfold from diabetic animals that were receiving vehicle. Opioid growth factor and opioid growth factor receptor distribution were comparable in diabetic and control animals. These results indicate a delay in reepithelialization that is dependent on the duration of diabetes and that intervention of endogenous opioid-receptor interfacing with an opioid antagonist can facilitate the process of wound healing. *Diabetes* 51: 3055–3062, 2002

Diabetes causes loss of vision not only from major abnormalities of the retina and lens but also from alterations in the tear film, eyelids, iris, ciliary body, cranial nerves, and the cornea (1,2). Patients with diabetes are at increased risk for developing corneal disorders such as epithelial defects, recurrent epithelial erosions, decreased sensitivity, delayed reepithelialization, abnormal wound repair, increased susceptibility to injury, increased epithelial fragility, ulcers, and edema (1,3–5). Other corneal alterations associated with diabetes in humans include changes in basement membrane thickness, hemidesmosome number, endothelial cell function, collagen, oxygen consumption and uptake, permeability to fluorescein, sensitivity, cell size, and deposition of complement proteins (4,6–10). The removal of the corneal epithelium of patients with diabetes during vitrectomy to restore corneal clarity temporarily has been found to be related to considerable difficulty with corneal reepithelialization and persistent epithelial defect/recurrent erosion in postoperative patients (1,4,11–13). In general, human and animal studies concur with clinical findings showing a decrease in the healing of the diabetic corneal epithelium (14–19).

Endogenous opioid peptides serve to regulate the growth of developing, neoplastic, renewing, and healing tissues and function in both prokaryotes and eukaryotes (20). One native opioid peptide, [Met^5]-enkephalin, termed opioid growth factor (OGF), has emerged as a receptor-mediated growth factor; this peptide is encoded by the preproenkephalin A gene. OGF is a potent, reversible, species- and tissue-nonspecific peptide that is a negative growth regulator. This peptide is autocrine and possibly paracrine produced, secreted, and effective at concentrations consistent with growth effects and the binding affinity of its receptor, OGFr. OGFr has been cloned and sequenced in humans and rodents and bears no resemblance to classical opioid receptors (20). Blockade of OGF–OGFr interaction with the use of opioid antagonists such as naltrexone (NTX), neutralization with antibodies to OGF, and antisense experiments with OGFr accelerate growth (20–24).

The relationship of opioid peptides and diabetes has received some attention (25–34). For example, high plasma levels of [Met^5]-enkephalin (28,29) but normal levels of β-endorphin (25) have been reported in patients with diabetes. Moreover, elevated levels of [Met^5]-enkephalin also have been recorded in genetically diabetic (*db/db*) mice (30,31), and prodynorphin peptides have been reported to be elevated in the brain of diabetic rats (34). Finally, the diabetic condition has been documented to be accompanied by diminished nociception and an exaggerated antinociceptive effect from exogenously administered opioids (26,32,33).

Previous in vitro and/or in vivo studies in humans, rats, and rabbits have demonstrated that OGF–OGFr interactions play a role in homeostasis and reepithelialization of the cornea (21,22,35). An excess of OGF depresses cellular renewal and wound healing and targets DNA synthesis,

From the Departments of [1]Neuroscience and Anatomy, [2]Comparative Medicine, and [3]Ophthalmology and Pathology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania.

Address correspondence and reprint requests to Dr. Ian S. Zagon, Department of Neuroscience and Anatomy, H109, The Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA 17033. E-mail: isz1@psu.edu.

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ANOVA, analysis of variance; CCD, charged-coupled device; LI, labeling index; NTX, naltrexone; OGF, opioid growth factor; OGFr, opioid growth factor receptor; STZ, streptozotocin.

cell migration, and tissue organization. Persistent blockade of OGF–OGFr interfacing using the opioid antagonist NTX has the opposite action on growth, with an increase in DNA synthesis and cell migration, and the preservation of architectural organization recorded. OGF and OGFr are ubiquitous components of the vertebrate cornea (36), including humans.

The present study addresses the premise that blockade of endogenous opioid action related to growth restores repair processes of ocular surface epithelium under diabetic conditions. Using streptozotocin (STZ)-treated rats as a model system for diabetes, ocular surface epithelium of the cornea was abraded from inner limbal margin to inner limbal margin, and the animals were subjected to a continuous daily blockade of opioid-receptor interfacing by the potent and long-acting opioid antagonist NTX. These experiments assayed the size of the defect, rate of repair, and incidence of complete reepithelialization to examine whether NTX can facilitate wound healing of the diabetic cornea.

RESEARCH DESIGN AND METHODS

Animals and induction of diabetes. Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were bred at The M.S. Hershey Medical Center. Animals were housed under standard laboratory conditions; water and Purina 5010 Rodent Chow were continuously available. All investigations conformed to the regulations of the National Institutes of Health and the guidelines of the Department of Comparative Medicine of The Pennsylvania State University.

Insulin-dependent diabetes was induced by intravenous injection of 65 mg/kg STZ into 6-week-old (~100 g) rats (17,37,38) under anesthesia. STZ was prepared fresh by dissolving powder (Sigma, St. Louis, MO) in 0.01 mol/l sodium citrate (pH 4.5).

Blood glucose levels were monitored from the tail vein using a 25-g butterfly catheter and measured using glucose oxidase–impregnated strips and a Blood Glucometer (Accu-Check III, Boehringer Mannheim Diagnostics, Indianapolis, IN). Glucose levels of 350 mg/dl or more were considered to be the minimum blood glucose level compatible with a stable nontoxic diabetic state (14,33) and were found to be fourfold higher than controls within 1 week; glucose levels in STZ rats remained elevated throughout the experimental period. Hyperglycemic animals had urine glucose values of 2,000 mg/dl and consumed twofold more food and sixfold more water than control rats; body weights of hyperglycemic rats were subnormal (decreases of 18% or more) beginning 1 week after STZ injection. Histologic analysis revealed a complete degranulation of β-cells in the diabetic rats.

Corneal abrasions. The procedures for wounding and observation of repair followed those of Zagon et al. (22). In brief, with the use of a dissecting microscope (SZ-ET; Olympus, Tokyo, Japan) and a cold light source (Highlight 2000; Olympus), a 5-mm-diameter circle located in the center of the cornea was produced with a disposable dermatology skin punch (Acuderm, Ft. Lauderdale, FL); these wounds extended from limbal to limbal margin but did not include any limbal tissue. Wounds were made between 0830 and 0930 h or 1600 and 1700 h. These time points were chosen because previous studies (39) revealed no differences in labeling index between animals wounded in the morning or the afternoon. The encircled corneal epithelium was removed with a No. 15 Bard-Parker scalpel blade. Any animal that experienced bleeding, corneal opacities, ulcerations, inflammation, or infection was not included in the study. Only one eye was wounded in each animal; at least 4 weeks separated wounding right and left eyes. Antibiotic drops composed of trimethoprim sulfate and polymyxin B sulfate (Bausch & Lomb) were applied to the eye after surgery.

Corneas of diabetic (D) and control (nondiabetic) (C) rats were abraded 1, 4, and 8 weeks after confirmation of a diabetic state. At 4 weeks, diabetic rats were divided into two groups receiving either intraperitoneal saline (DS) or 30 mg/kg NTX HCl (NTX) (DN) twice daily at 0800 and 1600 h; nondiabetic rats received saline (CS). At 8 weeks, both diabetic and nondiabetic rats were divided into two groups receiving i.p. saline (DS, CS) or 30 mg/kg NTX twice daily (DN, CN) at 0800 and 1600 h.

Photography. For photographing the wounded eyes, animals were anesthetized in a chamber attached to a halothane vaporizer and the residual epithelial defect was stained with topical fluorescein (Fluor-I-Strip, Ayerst Laboratories, Philadelphia, PA). With the use of an Olympus dissecting microscope with a tungsten light source and a gelatin Wratten #47 filter attached to a Sony charged-coupled device (CCD) camera, images were captured at 1.5× magnification. Photographs of at least six rat eyes per group were taken immediately after abrasion (0 h) and 16, 24, 32, 40, 48, 56, and 64 h after wounding. No animal was photographed at intervals <12 h so as to prevent disruption of healing by fluorescein application. Abraded animals were followed every week for 4 weeks using fluorescein after completion of wound closure to inspect for defects in healing. The area of defect was determined using Optimas and was calculated as the percentage of residual epithelial defect.

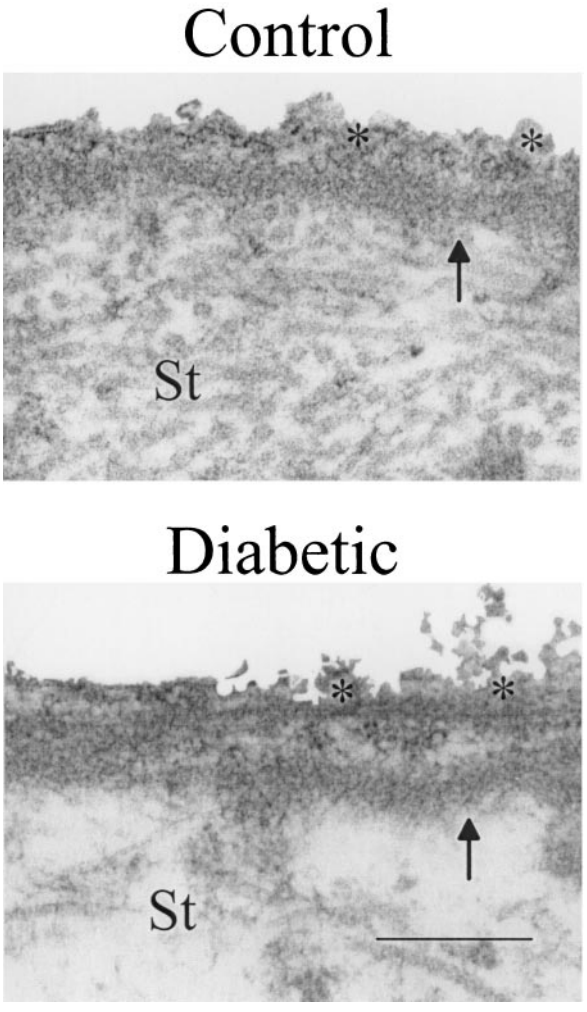

FIG. 1. Ultrastructural preparation of the ocular surface of control and 4-week diabetic rats immediately after corneal wounding. Abrasion of the epithelium removed the cell layers (*remaining cellular debris), but the basement membrane (arrows) remained intact. St, stroma. Bar = 700 nm.

Light and electron microscopy of wound healing. For verifying the injury and determining the magnitude of the defect created, both the injured and noninjured (control) corneas were collected immediately after wounding a 4-week diabetic animal. Light microscopy was used to ensure that the 5-mm wound did not include limbus and/or conjunctival epithelium, as well as to determine the depth of the wound. Electron microscopy was used to examine whether the basement membrane remained intact after abrasion. For light microscopic analysis, tissues were placed in 10% neutral buffered formalin and processed and embedded in paraffin, and sections were stained with hematoxylin and eosin or periodic acid-Schiff. For electron microscopy, corneas were fixed by immersion in a solution of 2% glutaraldehyde, 2.5% paraformaldehyde, 3% sucrose, and 0.025% $CaCl_2$ in 0. 1 mol/l sodium cacodylate buffer at 4°C for 18 h, postfixed in 1% OsO_4 for 2 h, and embedded in Epon 812. Thin sections of the abraded region were stained with 2% uranyl acetate and 0.4% lead citrate and viewed with a Philips 400 electron microscope (Philips Electronics Instruments, Mahwah, NJ).

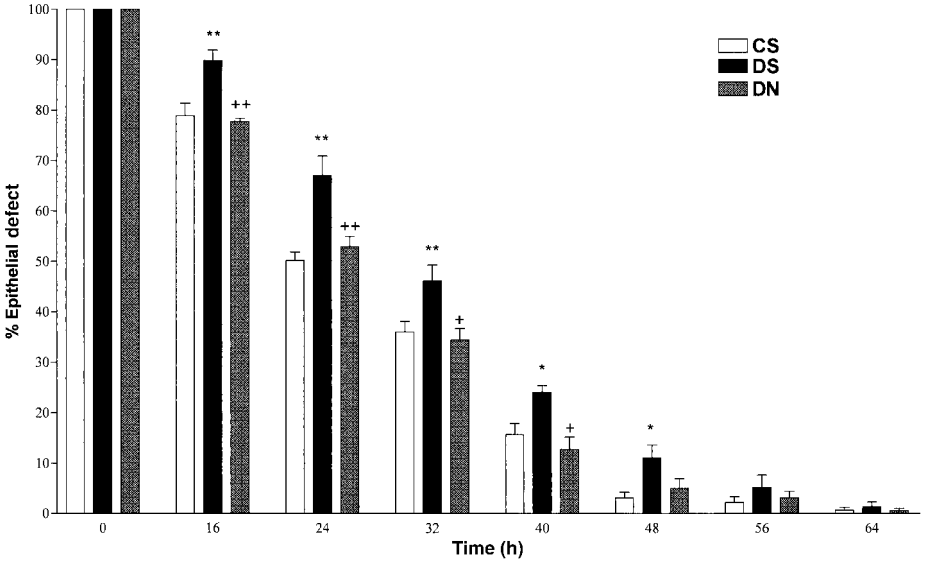

FIG. 2. Histogram of residual epithelial defect (%) in rat corneas after formation of a 5-mm corneal wound followed for 64 h. Three groups of rats were included: controls (nondiabetic) receiving saline (CS), 4-week diabetic rats receiving saline (DS), and 4-week diabetic rats given 30 mg/kg NTX twice daily (DN). Photomicrographs of the fluorescein-stained corneas were captured with a Sony CCD camera, and areas were analyzed by Optimas software. Residual epithelial defects are presented as percentage of the original wound. Data are expressed as means ± SE. No differences were recorded between the CS and DN groups. Significant difference between the DS and CS groups at *$P < 0.05$ and **$P < 0.01$. Significant difference between the DS and DN groups at +$P < 0.05$ and ++$P < 0.01$.

DNA synthesis. Procedures for measurements of DNA synthesis activity in the corneal epithelium followed those reported previously (40). In brief, 1 h before being killed (1600 h) uninjured animals from each experimental group (normal, diabetic, NTX-treated diabetic) received an i.p. injection of 2 μCi/g body wt [^{3}H]thymidine (20 Ci/mol, Du Pont-New England Nuclear, Boston, MA). Rats received a single i.p. injection of NTX (30 mg/kg) or an equivalent volume of sterile water at 1200 h; diabetic animals had received STZ 9 weeks earlier. The rats were anesthetized and decapitated, and eyes were proptosed and enucleated and processed for autoradiography. Eight-microgram sections that included the entire corneal surface, limbus, and conjunctiva were collected, coated with Kodak NTB-2 emulsion, stored in light-tight boxes at 4°C for 30 days, and developed with Kodak D-19. Tissues were counterstained with hematoxylin and eosin.

The number of cells with three or more grains (background was ≤1 grain/cell) in the basal epithelial layer of the central cornea, peripheral cornea, limbus, and conjunctiva was counted from two nonserial sections per eye, with four corneas assessed per group. Areas of analysis followed those described in Zagon et al. (40). At least 400 cells were examined in each region of the ocular surface. Only cells in the deepest aspect of the basal epithelium, situated proximal to the basement membrane, were considered basal cells. Labeling indexes (LIs) were computed as the number of labeled basal cells divided by the total number of basal cells with nuclei × 100.

Data analysis. All studies were conducted in a masked manner, and the same individuals performed the surgery and the morphometric analysis. Body weights, blood glucose levels, urine glucose and ketone levels, and food and water consumption were analyzed by *t* tests using Prism software.

The area of defect was analyzed at each time point using analysis of variance (ANOVA) and Newman-Keuls tests. The rate of healing was calculated between 0 and 24 h; the rate of wound healing is not linear (22); thus, linear regression over the entire period is inappropriate. The rate was calculated by dividing the total area that was reepithelialized by the number of hours elapsed. The number of corneas that were completely reepithelialized at a given time was compared using χ^2 tests. Data for the LIs were analyzed using ANOVA, and subsequent comparisons made with Newman-Keuls tests.

Immunocytochemistry. Immunocytochemical studies were performed on unwounded diabetic (4 weeks after confirmation of diabetes) and control rats to ascertain the distribution of OGF and OGFr in hyperglycemic animals. The immunocytochemical procedures, as well as the characteristics of the antibodies, have been described in previous studies (36). Primary antibodies to OGF (CO-172) and OGFr (I0028; fusion protein) were generated in our laboratory and used at concentrations of 1:250; secondary antibodies were used at dilutions of 1:100. Tissues were observed using an Olympus BH-2 microscope equipped with fluorescent, bright-field, and phase optics. Some sections served as controls and were incubated with secondary antibody only or with primary antibodies preabsorbed with either an excess of OGF or excess OGFr-fusion protein.

RESULTS

Corneal wounding in diabetic rats. The 5-mm trephine demarcated the entire corneal region of the rat eye but did not encroach on the limbus or conjunctiva (data not shown). At the light microscopic level of resolution, this method of debridement seemed to remove all cell layers constituting the corneal epithelium but preserved the basement membrane according to periodic acid-Schiff staining. These results were confirmed by electron microscopy of diabetic and control specimens (Fig. 1).

Corneal reepithelialization in diabetic rats. A total of 221 eyes were wounded in these studies: 100 diabetic corneas and 121 control corneas. Wound healing occurred in a manner consistent with previous studies on normal rat, rabbit, and human (21,22,35), with a leading edge emerging in a convex manner. The initial area of the abrasion ranged from 19.3 mm^2 to 20.4 mm^2 and corresponded to corneal injuries of 4.9 to 5.1 mm in diameter. No differences in the size of the initial abrasion were noted between diabetic and control rats.

Wound healing in the 1- and 4-week diabetic rat. No differences in corneal reepithelialization were observed between the diabetic and control animals at 1 week after confirmation of diabetes. Wound healing was delayed in the corneas of rats that were hyperglycemic for 4 weeks (Fig. 2). Significant delays in wound healing were noted in the DS group as compared with the CS group at 16, 24, 32, 40, and 48 h. Within the first day after abrasion, the DS group demonstrated retardation in reepithelialization and had significantly larger wounds than CS rats at 16 h (14%) and 24 h (23%). By 40 h, when the control wounds had a 15% defect, diabetic rats had ~25% residual wound. At 16, 24, 32, and 40 h, the NTX-diabetic rats (DN group) had

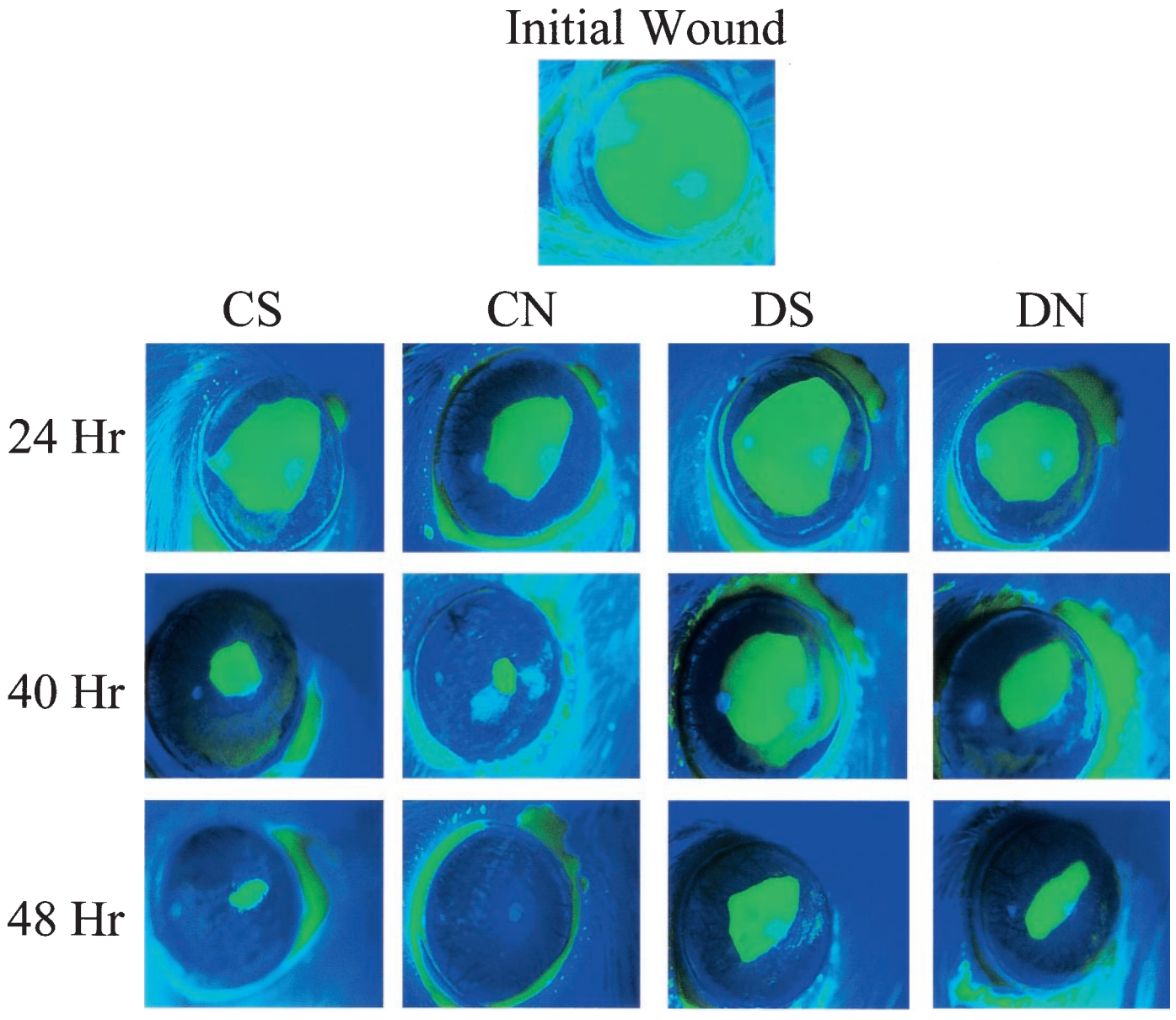

FIG. 3. Photographs of the rat eye stained with fluorescein immediately after (initial wound) or 24, 40, or 48 h after creation of a 5-mm corneal abrasion. Rats were either control (nondiabetic; C) or diabetic for 8 weeks (D) and received an i.p. injection twice daily of either 30 mg/kg NTX (N) or an equivalent volume of sterile saline (S). Magnification ×1.5.

significantly smaller wounds than the DS group, with a progressively greater difference noted between groups as healing occurred (e.g., a difference of 10% could be recorded at 16 h, whereas a difference of 47% was noted at 40 h). Determination of differences in wound size after 48 h was difficult to detect because of the small size of the defect. No differences in reepithelialization were evident between rats that composed the CS and DN groups at any time point. With regard to the incidence of complete wound closure, no differences were noted between any of the three groups at 48, 56, or 64 h.

Analysis of healing rates between 0 and 24 h revealed that the CS group had a rate of reepithelialization of 0.36 ± 0.01 mm^2/h, whereas the DS and DN groups had rates of 0.23 ± 0.01 and 0.36 ± 0.01 mm^2/h, respectively. The difference in rates of reepithelialization between the CS and DS group, as well as the DS and DN groups, was statistically significant ($P < 0.001$), but comparison between the CS and DN groups revealed similar rates of wound healing.

Wound healing in the 8-week diabetic rat. After 8 weeks of hyperglycemia, the diabetic rats that were receiving saline had wounds that reepithelialized significantly more slowly than control counterparts at 16, 24, 32, 40, 48, and 56 h (Figs. 3 and 4). The DS rats exhibited subnormal reepithelialization that ranged from reductions of 11% at 16 h to 183% at 40 h. Exposure of diabetic rats to NTX markedly facilitated the process of wound healing in comparison with diabetic animals that were receiving saline at all time points examined. This difference in reepithelialization between NTX- and saline-treated diabetic rats ranged from 12% at 16 h to 2.5-fold at 56 h. When the diabetic (DN) and control (CN) rats that were given NTX were appropriately analyzed by ANOVA, these groups were similar in reepithelialization at all but 40 h ($P < 0.01$) and 48 h ($P < 0.001$). In nondiabetic rats, NTX also was

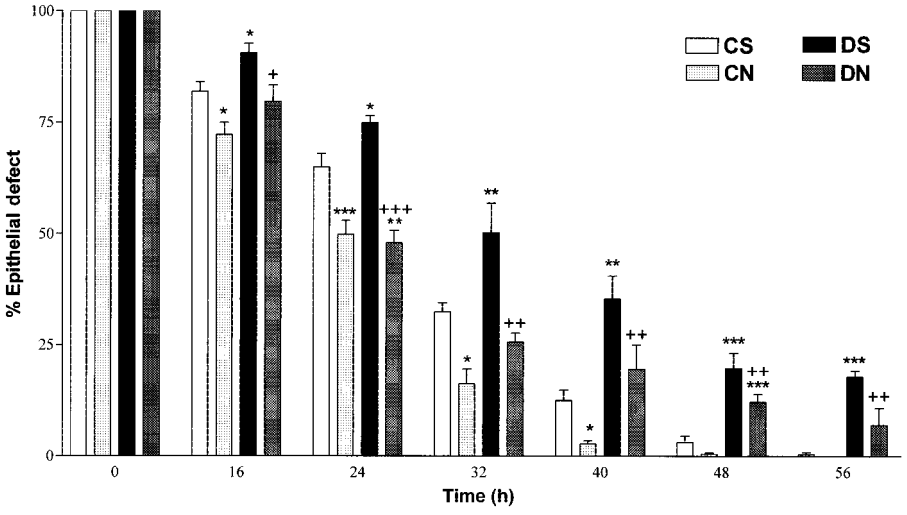

FIG. 4. Histogram of residual epithelial defect (%) in rat corneas after formation of a 5-mm corneal wound followed for 56 h. Rats were control (nondiabetic; C) or diabetic for 8 weeks (D) and received an i.p. injection twice daily of either 30 mg/kg NTX (N) or an equivalent volume of sterile saline (S). Photomicrographs of the fluorescein-stained corneas were captured with a Sony CCD camera, and areas were analyzed by Optimas software. Residual epithelial defects are presented as percentage of the original wound. Data are expressed as mean ± SE. Significantly different from controls (CS) at *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. Significant differences between the DS and DN groups at +$P < 0.05$, ++$P < 0.01$, and +++$P < 0.001$.

found to increase wound healing compared with control rats that received an injection of saline, with marked differences noted at 16, 24, 32, and 56 h that ranged from 12% (16 h) to 78% (40 h).

With regard to the incidence of complete wound closure, at 48 h after wounding, 67% of the nondiabetic rats that were receiving saline were healed in comparison with 0% of the diabetic rats that were receiving saline ($P < 0.02$); no differences were noted between DS and DN rats in terms of complete closure. At 56 and 64 h, no differences were noted in the incidence of closure; however, one rat in each of the CN, DS, and DN groups was not completely healed by 64 h.

Analysis of healing rates between 0 and 24 h revealed that the CS group had a mean rate of reepithelialization of 0.29 ± 0.01 mm^2/h, in comparison with the CN (0.39 ± 0.02 mm^2/h), DS (0.21 ± 0.01 mm^2/h), and DN (0.38 ± 0.02 mm^2/h) groups. Comparison of the rates of wound healing between the CS and DS groups, CS and CN groups, and CS and DN groups differed at the $P < 0.001$ level. No differences in the rate of reepithelialization were detected between the animals in the control and diabetic groups that were receiving NTX (CN and DN).

Overall healing rates of NTX-treated rats in both diabetic and nondiabetic groups at 8 weeks were different from their respective saline-treated groups. Linear regression indicated r values of 0.96 or higher for each of the treatment groups.

All groups of animals were followed on a weekly basis for a total of 4 weeks beyond complete wound closure. No residual or recurrent corneal epithelial defects were identified by fluorescein staining in the CS, CN, DS, or DN group.

DNA synthesis. The peripheral cornea, limbus, and conjunctiva of diabetic animals exhibited a subnormal number of cells undergoing DNA synthesis that ranged from 81 to 90% of control levels (Fig. 5). Administration of NTX for only the 4-h period to diabetic animals revealed increases of 4-fold, 3.5-fold, and 8-fold in the number of radiolabeled basal epithelial cells in the peripheral cornea, limbus, and conjunctiva, respectively. However, despite these increases in the number of cells recorded in DNA synthesis, the LIs in the peripheral cornea and the limbus of diabetic rats that were receiving NTX was subnormal. The conjunctiva of the control and diabetic rats that were given NTX were comparable in LIs. No cells labeled with radioactive thymidine were detected in the central cornea of animals in the control, diabetic, or diabetic/NTX groups.

Presence and location of OGF and OGFr in corneal epithelium of diabetic rats. Immunocytochemical analysis of the presence and distribution of OGF and OGFr in the corneal epithelium of diabetic rats was recorded 4 weeks after injection of STZ (Fig. 6). Both peptide and receptor could be observed in the cytoplasm but not in the nucleus in both control and diabetic specimens. Subjectively, there seemed to be little difference in staining intensity or distribution between groups with either OGF or OGFr antibodies. Preabsorbed control preparations (Fig. 6*B* and *D*) and those stained only with the secondary antibody showed little reactivity (data not shown).

DISCUSSION

With the use of a well-characterized model of diabetes in conjunction with established methodology in wounding of the corneal epithelium, a major finding in this study is that the integrity of the endogenous opioid system related to growth is maintained in the diabetic state. Evidence supporting this contention includes *1*) documentation of changes in repair of the corneal epithelium of diabetic rats challenged with the opioid antagonist NTX, *2*) interruption of opioid receptor interfacing in the diabetic rat facilitates reepithelialization in the direction predicted (i.e., acceleration), *3*) blockade of OGF–OGFr interaction by NTX in diabetic animals accelerates DNA synthesis of basal corneal epithelial cells, and *4*) the presence and appropriate location of OGF and OGFr, the principal opioid peptide and receptor involved with growth, in the ocular surface

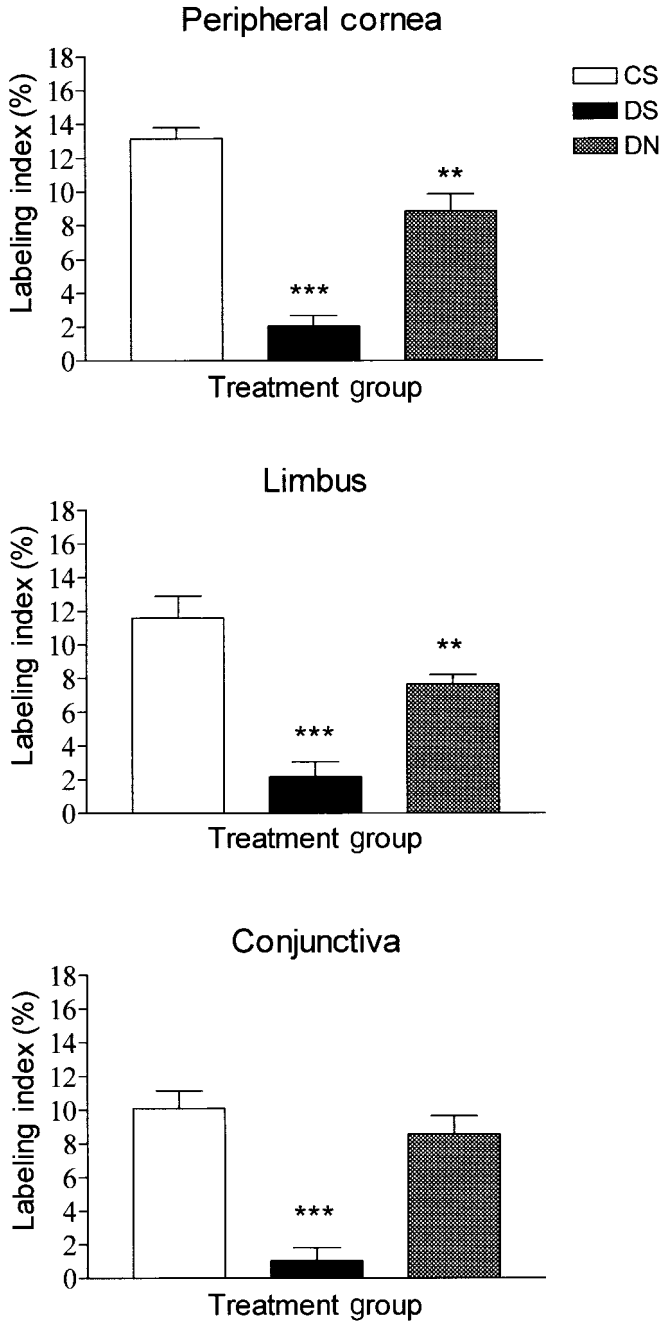

FIG. 5. Histogram of the LI of rat basal epithelial cells in three regions of the ocular surface; no labeled cells were found in the central cornea. Animals were control (nondiabetic; C) or 8-week diabetic (D). At 4 h before being killed, the control animals received an injection of saline (CS), whereas the diabetic animals were given injections of either NTX (DN) or saline (DS). One hour before being killed, all animals received [^{3}H]thymidine. Data represent mean ± SE. Significantly different from controls (CS) at $^{**}P < 0.01$ and $^{***}P < 0.001$.

epithelium of the diabetic animal. Therefore, one may conclude that although corneal wound healing is delayed in diabetes, the pathway of the endogenous opioid receptor involved with cell proliferation, migration, and differentiation continues to function in repair and presumably homeostasis.

Our study offers a second important advancement in our understanding of the biology of the cornea in showing that opioid antagonist treatment can increase corneal repair even under diabetic conditions, thereby extending our earlier knowledge about opioids and the corneal epithelium in normal animals. As observed previously in rats, humans, and rabbits (21,22,35) under in vitro and in vivo conditions, NTX exposure of animals or corneas in organ culture had an increase in reepithelialization, the rate of healing, and the incidence of complete wound closure. NTX seemed to have a greater effect on reepithelialization in diabetic animals compared with nondiabetic subjects at 8 weeks of hyperglycemia. For example, at 24 and 32 h, wound sizes in the CN and DN groups were similar. However, because the magnitude of the defect in diabetic animals was significantly greater than in nondiabetic rats, this suggests that the compensatory effect of NTX was more robust in the DN group than in the CN group. Because the influence of diabetes on corneal wound repair was less substantial at 4 weeks of hyperglycemia, comparison of the reparative effects of NTX between diabetic and nondiabetic animals was inconclusive.

The relationship of opioid peptides to diabetes has received some notice (25–34). Studies concerned with circulating opioid levels in diabetes have shown that patients with type 1 diabetes do not demonstrate any significant change in β-endorphin plasma levels (25), whereas elevated plasma levels of [Met^5]-enkephalin have been observed (28,29). Elevated levels of [Met^5]-enkephalin also have been reported in genetically obese diabetic (*db/db*) mice (30,31). Finally, a diminished nociception and an exaggerated antinociceptive effect from exogenously administered opioids have been recorded in diabetic animals (26,32,33). This information suggests that diabetes is accompanied by an elevated level of the opioid peptide [Met^5]-enkephalin.

Because [Met^5]-enkephalin serves as an inhibitory growth factor (i.e., OGF), one might conjecture that cell replication in diabetics is subnormal, including regions such as the corneal epithelium that undergo both cellular renewal and repair. Indeed, diabetic animals were found to have marked reductions in DNA synthesis in the basal epithelium composing the peripheral cornea, limbus, and conjunctiva, suggesting that cell replication in the homeostatic corneal surface is compromised under diabetic conditions. Administration of NTX notably increased DNA synthesis of ocular surface epithelium in diabetic rats and, in at least one region (conjunctiva), returned cell proliferation to normal levels. The absence of DNA synthesis in the central corneal epithelium in these studies supports similar findings reported elsewhere (39,40). A review of the literature demonstrates that glucose exposure can elevate (41,42), inhibit (43,44), or stimulate and then repress (45,46) cell proliferation. The only related paper found on the subject of glucose and cell proliferation of the corneal epithelium showed an increase in SV40 transformed human corneal epithelial cells in culture (41). The discrepancy in results between the present study and that of McDermott et al. (41) is not clear but may be related to differences under in vivo and in vitro conditions, the use of SV40-transformed human epithelial cells and nontransformed system, a paradigm of exposure to glucose and a model of diabetes, and/or the length of time of hyperglycemia. Because cell proliferation is an essential step in the healing process (39), it might be speculated that compro-

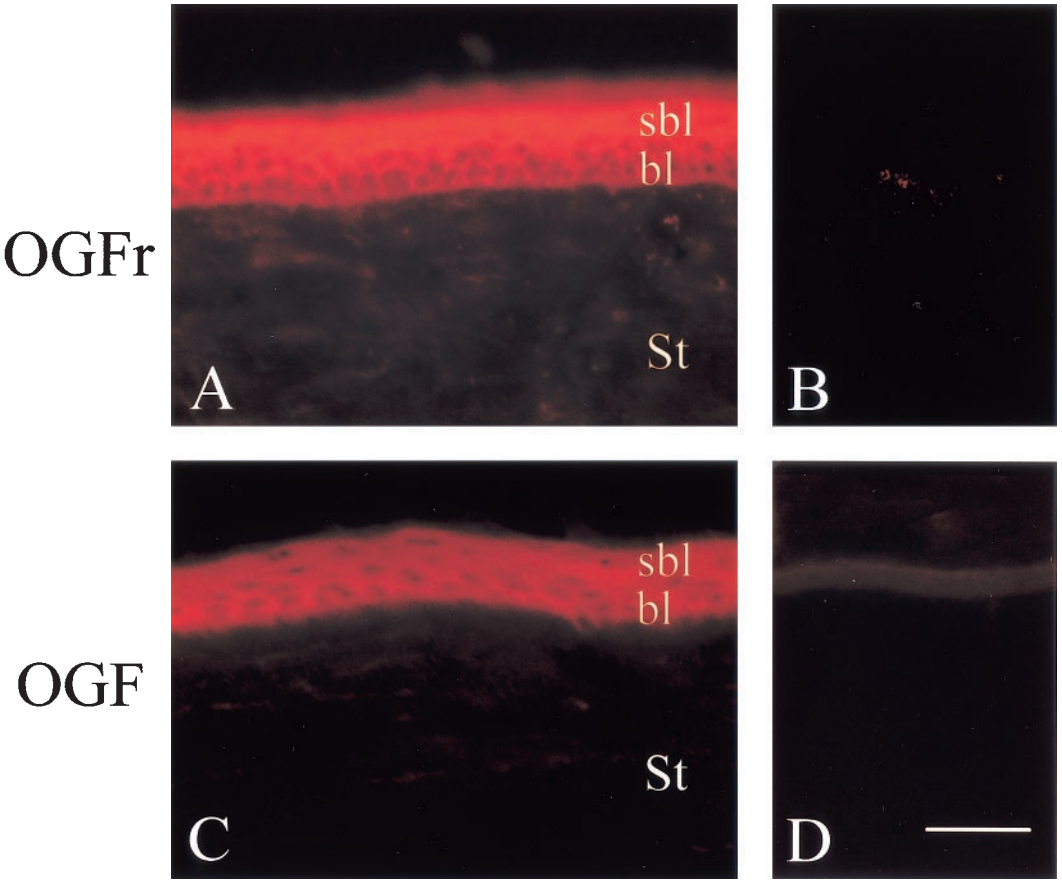

FIG. 6. Photomicrograph of immunocytochemical preparations of 4-week diabetic peripheral corneas stained with antibodies to OGFr (*A* and *B*) or OGF (*C* and *D*). Immunoreactivity for both OGFr and OGF was observed in control and diabetic specimens. Preparations stained with antibodies to OGFr or OGF that were preabsorbed with OGFr (*B*) or OGF (*D*) antigen have been included. St, stroma; bl, basal epithelium; sbl, suprabasal epithelium. Bar = 40 μm.

mising of DNA synthesis under diabetic conditions may contribute to the impaired wound healing in diabetes. Moreover, our findings suggest that use of a blockade of the OGF–OGFr pathway could be valuable in ameliorating the deficits in cell replication by preventing OGF's repressive activities on cellular repair.

Examination of corneal reepithelialization in the face of the diabetic state revealed a number of intriguing features of this disease. During the first week of a hyperglycemic conditions, wound healing of the corneal surface was not disturbed. These results suggest that alterations in reepithelialization of the ocular surface are not dependent on hyperglycemia and/or accompanying features, at least initially. It was apparent that there was a progressive delay expressed in the healing process of the ocular surface epithelium that was time dependent with respect to the diabetic state. Thus, at 4 and 8 weeks, comparison of the diabetic and control subjects showed marked delays in the repair of the corneal epithelium. It also was noted that the magnitude of these delays in reepithelialization were similar in the 4- and 8-week diabetic groups, indicating little difference in this respect whether the duration of hyperglycemia was 1 or 2 months. The results in the present article stand in agreement with those of other studies showing a decrease in the healing of the corneal epithelium in diabetic animals (14–16), although Friend et al. (16) and Hatchell et al. (19) reported more rapid reepithelialization in diabetic rabbits and Snip et al. (47) described a lack of change in corneal epithelial regeneration in diabetic patients. Our data in animals with the animal model established also can be correlated with delayed wound healing in humans with diabetes (48,49), and especially with reference to the ocular surface epithelium (4,12).

Given that the ocular surface epithelium is vital to the visual process and that patients with diabetes are at risk for increased problems with corneal disorders (e.g., persistent corneal defects, recurrent erosion), which can cause serious complications after some ophthalmic surgical procedures (e.g., vitrectomy), the present data offer exciting potential for therapeutic intervention. Thus, our findings may suggest an important and novel clinical use for opioid antagonists such as NTX in rescuing the dysfunctional corneal epithelium in patients with diabetes. As a corollary, administration of opioid antagonist therapy may offer a strategy to protect the corneal epithelium in hyperglycemic patients and thereby restore function to homeostasis and healing processes.

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