The opioid growth factor, [Met⁵]-enkephalin, inhibits DNA synthesis during reepithelialization of mouse tail skin

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Abstract. Opioid peptides serve as tonically active negative growth regulators in renewing and regenerating epithelia. To examine the involvement of opioids in renewal of the stratum corneum after tape stripping of tail skin, C57BL/6 J mice were given systemic injections of the potent opioid antagonist, naltrexone (NTX, 20 mg/kg i.p.) following injury. Blockade of opioidreceptor interaction by NTX for 4 h resulted in an elevation of 36–66% in basal cell DNA synthesis measured 24 h after injury. Injection of the endogenous opioid peptide, [Met⁵]-enkephalin (OGF, 10 mg/kg i.p.) 4 h before termination, suppressed radiolabelled thymidine incorporation in the basal cell layer by 37–46% at 24 h after wounding. The magnitude of the effects on DNA synthesis of OGF, but not NTX, depended on the timing of administration with respect to injury. OGF maximally depressed basal cell labelling (72%) when given 16 h after tape stripping. Concomitant administration of naloxone (10 mg/kg) with OGF blocked the inhibition of DNA synthesis; naloxone alone at the dosage utilized had no effect on cell labelling. Both OGF and its receptor, OGF_r, were detected by immunocytochemistry in the basal and suprabasal cell layers, but not the cornified layer of tape stripped and uninjured tail skin. These results indicate: (a) a native opioid peptide and its receptor are expressed in epidermal cells of injured and uninjured mouse tail skin; (b) removal of the stratum corneum by tape stripping does not disrupt the function of the endogenous opioid growth system; (c) the proliferative response to wounding of the tail is tonically inhibited by the receptor-mediated action of an endogenous opioid peptide; and (d) DNA synthesis by basal cells can be elevated by disrupting opioid peptidereceptor interactions.

INTRODUCTION

A number of substances have been identified as growth factors in homeostasis and healing of the skin (see reviews: McGrath 1990, Martin 1997). The majority of these substances appear to be positive regulatory factors (e.g. epidermal growth factor (EGF), transforming growth factor (TGF)- α , and platelet-derived growth factor (PDGF)). With respect to the highly

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organized cellular hierarchy of the epidermis, it has been suggested that a 'proliferative inhibitor' produced within the epidermal cell population, may function in regulating basal cell proliferation (Bullough 1962, Potten & Allen 1975, Potten 1975, Marks & Richter 1984, Potten 1986). In the past decade, endogenous opioid peptides have emerged as tonically active, negative regulators of growth in developing, neoplastic, renewing, and healing tissues (Zagon & McLaughlin 1983a,b, McLaughlin, Levin & Zagon. 1999). One native opioid peptide, [Met⁵]-enkephalin, interacts with a receptor to suppress growth (Zagon & McLaughlin 1991, Zagon, Wu & McLaughlin 1983b, Schmal *et al.* 1989, Davila-Garcia & Azmitia 1990, Steine-Martin & Hauser 1990, Zagon & McLaughlin 1991, McLaughlin 1994, Zagon, Wu & McLaughlin 1996b, Zagon, Sassani & McLaughlin 1998, Zagon, Sassini & McLaughlin *et al.* 1999, Zagon & McLaughlin 1993a). To distinguish the role of this peptide as a growth factor in neural and non-neural cells and tissues, and in prokaryotes and eukaryotes, [Met⁵]-enkephalin, has been termed opioid growth factor (OGF) (Zagon & McLaughlin 1991). Unlike other opioid receptors, the function (e.g. growth), molecular organization, distribution (i.e. neural and non-neural tissues), transient appearance during ontogeny, ligand specificity (i.e. [Met⁵]-enkephalin), competitive inhibition profile, and the fact that ligands for other known opioid receptors (e.g. μ , δ and κ) do not influence growth, have provided a unique set of characteristics that distinguish the OGF receptor (OGFr; formerly termed the ζ receptor; Zagon, personal communication) from other classical opioid receptors (Zagon & McLaughlin 1991, Zagon & McLaughlin 1993a).

With respect to epithelium, homeostatic renewal of this tissue in murine tongue (Zagon, Wu & McLaughlin 1994a,b), gastrointestinal tract (Zagon, Wu & McLaughlin 1997), skin (Zagon *et al.* 1996b) and cornea (Zagon *et al.* 1997) is governed by OGF. Moreover, both OGF and OGFr have been localized by immunocytochemistry in human and mouse skin, including the epidermis of the rodent tail (Zagon *et al.* 1996b). Recent studies have demonstrated that OGF modulates re-epithelialization of the mammalian cornea following injury (Zagon *et al.* 1998).

Because opioids regulate cell proliferation during homeostatic renewal and repair in a variety of epithelial structures, it was theorized that opioid peptides also may control basal epidermal cell proliferation during regeneration of the stratum corneum following injury. Removal of this layer of cornified cells by repetitive tape stripping induces rapid and vigorous proliferation of basal cells of the epidermis (Pinkhus 1951, Potten & Allen 1975, Stoscheck, Nanney & King 1992, Wilson *et al.* 1998). In the present study, tape stripping of the mouse tail was chosen as a model system to explore our hypothesis because the method of wounding is confined to the epidermis (Pinkhus 1951), and the tail is accessible and relatively hairless compared to back skin (Stoscheck *et al.* 1992, Wilson *et al.* 1998). Furthermore, since the effect on basal cell turnover by OGF is circadian-dependent in some epithelium (Zagon *et al.* 1994a,b, Zagon *et al.* 1996b), the mouse tail was selected because of minimal circadian variation in homeostatic DNA synthesis of the epidermis (Potten 1975, Wilson *et al.* 1998).

A number of experimental paradigms were employed in this study to determine if an endogenous opioid system is operative during recornification of injured mouse tail skin. First, opioid receptor blockade with the opioid antagonist, naltrexone was used to investigate whether OGF and OGFr are functional constituents of the proliferating basal cell population following injury. Second, exogenous OGF was given to mice to determine if this peptide influences regeneration of the stratum corneum. Third, the receptor-mediated mechanism of OGF as to recornification of the epidermis was ascertained. Fourth, evaluation of the presence of OGF and OGFr in wounded and unwounded epidermis was undertaken using immunocytochemistry.

MATERIALS AND METHODS

Inbred C57BL/6 J specific pathogen-free male mice (The Jackson Laboratory, Bar Harbor, ME, USA), 7–9 weeks in age, were used in this study. Mice were housed in solid bottom metal cages (3 mice per cage) in an environment of 21 ± 0.5 °C with a relative humidity of $50 \pm 10\%$. The room had a complete exchange of air 15–18 times/h and a 12:12-h light–dark cycle (light from 07.00 to 19.00 h) with no twilight. Food (Teklad Rodent Diet #8604, Harland-Teklad, Madison, WI, USA) and chlorinated water were provided *ad libitum*.

The method of wounding the tail was a modification of procedures reported by Stoscheck *et al.* (1992) in which hair was not removed prior to tape stripping. Mice were anaesthetized by intraperitoneal (i.p.) injection of ketamine (Ketaset®, Fort Dodge Laboratories Inc., Fort Dodge, IA, USA; 100 mg/kg) and xylazine (Xyla-Ject®, Phoenix Pharmaceutical Inc., St Joseph, MO, USA; 7 mg/kg). Cloth tape (Durapore®, 3M Corp., Minneapolis, MN, USA), 1 cm wide, was wrapped around the tail 1 cm from its base. The tape was removed immediately and this process repeated for a total of 10 times. Wounds were created between 08.00 and 09.00 h and the animals terminated 24 h later. All experiments with mice were carried out with the approval of the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine.

Four sets of experiments were conducted. In the first, mice were injected i.p. with OGF ([Met⁵]-enkephalin, Sigma Chemical Co., St Louis, MO, USA; 10 mg/kg); the long-acting opioid antagonist, naltrexone (NTX, Sigma; 20 mg/kg); or an equal volume of sterile phosphate buffered saline (PBS, vehicle) at 05.00 h. A separate group of mice were wounded but were not injected with drug or vehicle. Thirty minutes prior to termination 24 h after wounding and 4 h of drug exposure, animals were given an i.p. injection of [³H]-thymidine (5 µCi/g body weight, specific activity 20 Ci/mmol.; DuPont–New England Nuclear, Boston, MA, USA).

In the second set of experiments, mice were injected i.p. with either OGF (10 mg/kg), OGF (10 mg/kg) and naloxone hydrochloride (NAL, Sigma; 10 mg/kg), NAL (10 mg/kg) alone, or an equivalent volume of sterile PBS vehicle at 05.00 h. Thirty minutes prior to termination 24 h after wounding at 09.00 h, and after 4 h of drug exposure, animals were given an i.p. injection of [³H]-thymidine.

In a third set of investigations, mice were injected i.p. with OGF (10 mg/kg) or an equivalent volume of sterile PBS at 09.00 h, 17.00 h, 21.00 h, 01.00 h or 07.00 h after wounding at 09.00 h, i.e. drug exposure of 24, 16, 12, 8, or 2 h, respectively. Thirty minutes prior to termination 24 h after wounding, animals were given an i.p. injection of [³H]-thymidine.

In the fourth set of experiments, mice were injected i.p. with NTX (20 mg/kg) or an equivalent volume of sterile PBS at 09.00 h, 17.00 h, 21.00 h, 01.00 h, 05.00 h or 07.00 h after wounding at 09.00 h, i.e. drug exposure of 24, 16, 12, 8, 4 or 2 h, respectively. Thirty minutes prior to termination 24 h after wounding, animals were given an i.p. injection of [³H]-thymidine.

Mice were sacrificed by cervical dislocation. The entire tail of each mouse was removed and immersed in 10% neutral buffered formalin for 24 h. After fixation, the dorsal skin of the tail was removed by making a longitudinal incision on both sides of the tail and stripping the skin from the underlying fascia. The tail skin was trimmed so that only the wounded region was processed. Paraffin-embedded tissue was sectioned perpendicular to the surface at 8 µm, and sections coated with NTB-2 emulsion (Eastman Kodak Co., Rochester, NY, USA) and stored in light-tight boxes at 4°C. After 17–19 days of exposure, the tissues were developed

with Kodak D-19 (Eastman Kodak) and counterstained with Mayer's haematoxylin and eosin. The number of cells (at least 500 cells/section) with 4 or more grains (background was 1 grain/cell) in the basal layer was counted from 2 sections/mouse at each time point. Only cells in the deepest aspect of the basal epithelium (i.e. adjacent to the basement membrane) were evaluated. The interfollicular epidermis was evaluated and the hair follicles were avoided. At least two mice were examined at each time point.

Labelling indices (LI) were computed as the number of labelled basal cells divided by the total number of basal cells with nuclei $\times 100$. Data were analysed using analysis of variance (ProStat, Poly Software International, Salt Lake City, UT, USA), and subsequent comparisons made using Newman-Keuls tests.

To examine the presence of the OGF and its receptor, OGF_r, dorsal tail skin was removed from one mouse 24 h after tape stripping and frozen in isopentane and dry ice. Ten-micrometre sections were collected on gelatin-coated slides and air-dried. Tissues were fixed 10 min in 95% ethanol and 10 min in acetone at -20°C , rinsed in Sorenson's phosphate buffer and 3% normal goat serum and 0.1% Triton X-100 at room temperature for 15 min. Ammonium sulphate-purified anti-OGF IgG or anti-OGF_r IgG was diluted (1:100) in Sorenson's phosphate buffer with 1% normal goat serum and 0.1% Triton X-100. Details about the production and characteristics of the antibodies to [Met⁵]-enkephalin (CO-172) and OGF_r (AO-440) have been reported elsewhere (Zagon & McLaughlin 1993b, Zagon *et al.* 1996b). Tissues were incubated with the primary antibodies in a humidified chamber at 4°C for 20 h, washed and incubated with rhodamine-conjugated goat anti-rabbit IgG (1:100; ICN Biomedical, Costa Mesa, CA, USA) for 45 min. Images were captured with a three-chip Sony CCD camera (model DKC-5000) mounted on an Olympus BH-2 light microscope equipped with rhodamine interference filters.

RESULTS

Tape stripping of the mouse tail stimulated basal cell proliferation in the epidermis. Twenty-four hours following injury at 09.00 h, the LI of basal epithelial cells from mice not receiving drug or vehicle was $14.8 \pm 1.5\%$. This level of radiolabelled thymidine incorporation was comparable to the LI ($12.2 \pm 2.7\%$) detected 24 h after injury in mice injected with sterile PBS 4 h prior to sacrifice. Furthermore, basal cell DNA synthesis in mice given PBS at 0, 8, 12, 16, 20, and 22 h after tape stripping and examined 24 h post-injury did not differ from either of these values (mean LI $12.8 \pm 0.7\%$).

The effects of opioid receptor blockade on DNA synthesis of basal cells in the epidermis of injured tail skin were determined (Figures 1 and 2). The LI of the basal epithelium 24 h after injury was 1.4-to 2.5-fold greater in mice injected with NTX 20 h after tape stripping compared to control values. In contrast, exposure to OGF for 4 h before termination at 24 h after wounding decreased DNA synthesis in basal epidermal cells by 45% to 63% from control levels (Figures 1 and 3). This reduction of the LI of basal epithelial cells was blocked by concurrent administration of the short-acting opioid antagonist, NAL (Fig. 2). Mice receiving only NAL 4 h prior to termination at 24 h after tape stripping had an LI that was comparable to control specimens.

The time-dependence of the effects of OGF or NTX treatment over a 24-h period after wounding was studied (Figures 2 and 4). OGF administered as early as 8 h after tape stripping reduced basal cell labelling at 24 h post-injury by 40% from control levels. The optimal effect of OGF on radiolabelled thymidine incorporation by the basal epithelium

Figure 1. Histogram of the labelling index (LI) of cells in the basal layer of wounded dorsal tail epidermis from C57BL/6J mice. Specimens were evaluated 24 h after repetitive tape stripping. Mice were given either OGF (10 mg/kg; $n = 3$), NTX (20 mg/kg; $n = 3$), or an equivalent volume of PBS (control; $n = 3$) by i.p. injection 4 h before termination. [^3H]Thymidine was administered 30 min prior to sacrifice. At least 1500 cells/animal were counted. Error bars, mean $\pm$ SEM. **Significantly different from control value at $P < 0.01$.

was detected when the peptide was injected 16 h after tape stripping (subnormal by 72%). The magnitude of suppression of DNA synthesis observed subsequent to injection of OGF at this time point was significantly different from any other time of drug administration. Even exposure to OGF for as little as 2 h prior to termination 24 h after wounding, reduced basal cell labelling by 50% (Figure 4). OGF administered at the time of injury lowered the LI measured 24 h later by 23%, but was not significantly different from control levels.

NTX administered at any time point after injury increased the level of DNA synthesis by 18% to 36% in basal cells 24 h following tape stripping (Figure 2). No significant differences across time were noted for the stimulatory effects of NTX.

Antibodies to OGF and OGF r were used in immunocytochemical-stained preparations to determine the distribution and location of the growth-related peptide and its receptor in tail skin of mice (Figure 5). Both antibodies provided a similar pattern of immunocytochemical labelling in the tail epidermis. There was no apparent qualitative difference in the staining pattern between wounded and unwounded tail skin. The epidermis, but not dermis, stained intensely; including cells of the strata basale and spinosa. The stratum corneum was not immunofluorescent. Both OGF and OGF r appeared to be associated with the cortical cytoplasm; this was prominent in the strata basale and spinosa. Little immunofluorescence was associated with the cell nuclei. Control specimens of mouse skin processed with antibodies pre-absorbed with the appropriate antigen (i.e. OGF or OGF r), or samples stained with secondary antibody only, exhibited no immunoreactivity (reported previously Zagon *et al.* 1996b).

Figure 2. Histogram of the labelling index (LI) of cells in the basal layer of wounded dorsal tail epidermis from C57BL/6J mice. The LI of control animals was established at 100%. Specimens were evaluated 24 h after repetitive tape stripping. NTX (20 mg/kg; $n = 3/\text{time point}$) or an equivalent volume of PBS (control; $n = 3/\text{time point}$) was administered i.p. at various times before termination. [^3H]Thymidine was given 30 min prior to euthanasia. At least 1500 cells/animal were counted. Error bars, mean $\pm$ SEM. *Significantly different from control value at $P < 0.05$.

DISCUSSION

This study provides several lines of evidence that endogenous opioid peptides play a role in regulating DNA synthesis in epidermal cells following injury. First, the LI of basal epithelium was elevated in response to opioid receptor blockade by administration of NTX. This finding suggests that interference with opioid receptor interaction in the epidermis has a profound impact on regeneration of the stratum corneum following its removal. Because NTX, a pure antagonist to the biological activity of opioids and devoid of intrinsic activity (Zagon & McLaughlin 1991, Zagon & McLaughlin 1993a), elicited this increase in DNA synthesis, it may be concluded that naturally occurring opioid peptides restrain growth, and do so by a receptor-mediated mechanism. Second, administration of an exogenous opioid peptide, OGF, to mice after tape stripping markedly suppressed labelling of basal cells. This result confirms that opioid peptides operate as negative growth regulators in injury-stimulated epidermis. Third, concomitant administration of NAL blocked the inhibitory effect of OGF, corroborating the premise that opioid peptide regulation of DNA synthesis by basal cells is receptor-mediated. Fourth, immunocytochemical localization of OGF and OGF r in the cytoplasm of epidermal cells provide evidence that this opioid growth peptide functions at the cellular level in an autocrine and possibly paracrine manner. Together these findings demonstrate that an endogenous opioid growth system is constitutively expressed in the epidermis and acts to tonically inhibit basal cell proliferation during reepithelialization of the epidermis. These data extend the work of Zagon *et al.* (1996b) indicating that OGF and its

Figure 3. Histogram of the labelling index (LI) of cells in the basal layer of wounded dorsal tail epidermis from C57BL/6J mice. Specimens were evaluated 24 h after repetitive tape stripping. The LI of control animals was established at 100%. OGF (10 mg/kg; $n = 3$), NAL (10 mg/kg; $n = 3$), or OGF (10 mg/kg) and NAL (10 mg/kg; $n = 3$), or an equivalent volume of sterile PBS (control; $n = 3$) was administered i.p. 4 h before termination. [^3H]Thymidine was given 30 min prior to sacrifice. At least 1500 cells/animal were counted. Error bars, mean $\pm$ SEM. **Significantly different from control value at $P < 0.01$.

receptor, OGF r , are present in mouse skin and play an inhibitory role in both normal homeostasis and renewal of the stratum corneum. The present investigation also is in agreement with earlier reports demonstrating that opioids function as negative growth modulators in proliferating cell populations (Steine-Martin & Hauser 1990, Zagon & McLaughlin 1991, Zagon & McLaughlin 1993a, McLaughlin 1994, Zagon *et al.* 1994a,b).

Given that NTX is not selective for OGF r , but blocks opioid interaction with many receptor subtypes (e.g.), the question arises as to which opioid peptide(s) regulate growth. The opioid peptide, [Met 5]-enkephalin, known as OGF was used in this study because of its extensive characterization as a ubiquitous growth factor in developing, neoplastic, renewing and healing tissues of both neural and non-neural origin (Zagon & McLaughlin 1983a,b, Zagon & McLaughlin 1991, McLaughlin 1994, Zagon *et al.* 1996a,b, Zagon *et al.* 1998, McLaughlin *et al.* 1999, Zagon *et al.* 1999). It is possible that opioids in addition to OGF could participate in the regulation of cell proliferation within the epidermis; this point requires further clarification. Nonetheless, this report presents evidence that at least one native opioid peptide, OGF, is present and plays a role in the recornification of the skin.

Although the duration of action of OGF in various tissues is unknown, plasma levels of exogenously administered [Met 5]-enkephalin decline after approximately 5 min (Akil *et al.* 1984). Interactions of the receptor, OGF r , with either agonist (OGF) or antagonist (NTX) induced a rapid cascade of events (within 2 h) resulting in altered cell proliferation. Thus, OGF injected as soon as 8 h after injury decreased radiolabelled thymidine incorporation, which persisted for at least 16 h after initiation of drug exposure. These results suggest that the manipulation of opioid-receptor interaction is rapid in onset and long-lasting. Maximum

Figure 4. Histogram of the labelling index (LI) of cells in the basal layer of wounded dorsal tail epidermis from C57BL/6J mice. Specimens were evaluated 24 h after repetitive tape stripping. The LI of control subjects was established at 100%. OGF (10 mg/kg; $n = 3$ /time point) or an equivalent volume of sterile PBS (control; $n = 3$ /time point) was administered i.p. at various times after tape stripping. [3 H]Thymidine was given 30 min prior to sacrifice. At least 1500 cells/animal were counted. Error bars, mean $\pm$ SEM. **Significantly different from control value at $P < 0.01$; †Significantly different from all other time points ($P < 0.01$).

suppression of DNA synthesis by basal cells in the present study was observed when OGF was given 16 h after injury. A possible explanation for the marked inhibition of labelling when stimulated epidermis is exposed to OGF for 8 h may lie with the effect of the peptide on basal stem cells. Potten (1975) introduced the concept of 'epidermal proliferative units' in which basal stem cells divide to produce a stem cell and non-stem cell, both capable of amplifying subsequent cell divisions. Thus OGF administered 16 h after injury could affect DNA synthesis in both basal stem and non-stem cells. In contrast, NTX-treatment elevated DNA synthesis in epidermal basal cells whether injected at the time of injury or at various times in between wounding and termination, indicating that even in states of increased cell turnover, endogenous opioids are continuously available and tonically active in controlling epidermal basal cell replication.

There are several possible explanations for why cell proliferation increases after tape stripping if the endogenous opioid system remains functional following injury. First, the concentration of endogenous OGF may decrease following injury, releasing the negative control on cell proliferation. Inhibition of DNA synthesis by injecting OGF suggests that negative regulation can be amplified by administration of exogenous peptide. Second, the receptor, OGFr, may decrease in number following wounding or become dysfunctional. Although immunocytochemical staining for the receptor was not quantitative, there was no apparent difference in staining pattern between wounded and unwounded tail skin. Furthermore, increased DNA synthesis after injection of NTX indicates that the receptor is

Figure 5. Photomicrographs of longitudinal sections of dorsal tail skin from C57BL/6J mice using brightfield (a,b) or indirect fluorescent (c–f) optics. Frozen sections (10 μ m) were obtained from the adjacent, unwounded (a,c,e) or wounded skin (b,d,f) 24 h after tape stripping. In panel (a), components of the epidermis (*epi*): stratum corneum (*sc*), stratum spinosum (*ss*), and stratum basale (*sb*); and the dermis (*de*) are identified. The stratum corneum was markedly reduced after tape stripping (b,d,f). Immunofluorescence was observed in the cortical cytoplasm (arrows) of the cells in the stratum spinosa and basale, but not the stratum corneum, using antibodies to OGF (c,d) or to OGFr (e,f). No immunoreactivity was recorded in the dermis. Scale bar: 28 μ m.

indeed functional. Finally, it is conceivable that other growth substances and receptors increase in relative importance following injury. Stoscheck *et al.* (1992) showed that the receptor for epidermal growth factor (EGF) increased in number, reaching a maximum at 96 h after tape stripping of mouse tail skin. Thus, the opioid growth factor system may play an important role during homeostasis and work in concert with other growth factors (EGF, TGF- α , PDGF) following injury. It is plausible that OGF and its receptor may play a prominent role at the end stages of repair as wound healing ceases and normal homeostasis returns. The present investigation focused on cell responses 24 h after injury. It is unknown how OGF and OGFr change over the duration of the repair process; i.e. beyond 24 h.

Changes in epidermal OGF and OGF α concentration and function at times beyond 24 h following tape stripping must be explored.

The present study focused on proliferation of the basal cell during recornification of the stratum corneum after tape stripping. These data, in concert with recent reports documenting OGF control of re-epithelialization of corneal wounds (Zagon *et al.* 1998, Zagon *et al.* 1999), lead to speculation on the clinical implications of the endogenous opioid growth system in tissue repair. To aid in understanding the clinical significance of an endogenous opioid system of growth regulation, it is of interest to speculate on the potential magnitude of effect on the healing process. Estimating the total contribution of OGF to epidermal repair needs to take into account several factors. First, the activity of endogenous OGF can be roughly calculated (assuming that OGF is the only endogenous opioid accounting for alterations in DNA synthesis) by data on the effects of opioid receptor blockade using NTX. Thus, at 16 and 20 h after injury, opioid antagonist exposure elicited changes in LI of 26% and 36%, respectively. Second, the capacity of exogenous OGF, presumably acting by saturating sites where endogenous peptide is absent, needs attention. Again, at 16 and 20 h following injury, administration of OGF decreased the LI by 72% and 63%, respectively. Summing the effects of endogenous and exogenous OGF activity at these time points, yielded accumulative effects of 98% to 99%. Moreover, these changes were observed after a one-time, short exposure (4 or 8 h) to OGF or NTX at one interval (24 h) after tape stripping. Additional investigations as to the significance of opioid activity on the entire healing process need to be explored.

To extend the present findings to clinical applications, it could be theorized that the endogenous opioid growth system may be involved in both normal and abnormal biological processes of the epidermis. In addition to the results reported herein, immunocytochemical studies have identified OGF and OGF α in human epidermis (Zagon *et al.* 1996b). Moreover, Nissen & Kragballe (1997) reported that OGF inhibited differentiation and proliferation of human keratinocytes *in vitro* and that naltrexone antagonized this inhibition. It is possible that overexpression of peptide or receptor, either at the transcriptional or translational levels, could result in reduction of the number of cells undergoing DNA synthesis and cellular content of skin. Such an event could play a role in age-related trophic changes in the epidermis or the pathogenesis of chronic wounds, delayed wound healing or other dysfunctions of the repair process. Similarly, under-expression of OGF and/or OGF α could explain the development of hyperplasia of the epidermis or altered turnover of keratinocytes observed in certain dermatopathologies (e.g. epidermolytic hyperkeratosis, ichthyosiform erythroderma, Vohlwinkel's syndrome). In light of recent reports of elevated [Met⁵]-enkephalin levels in psoriatic lesions (Nissen *et al.* 1997, Nissen *et al.* 1999), it is reasonable to postulate that OGF α may be absent, present in low number or non-functional in this and other pathologic states of epidermal hyperplasia with suspected psychogenic basis (e.g. exfoliative dermatitis, lichen planus, eczemas). Finally, blockade of opioid receptors by opioid antagonists such as NTX could prove to be of therapeutic value in modulating the wound healing process.

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