OPIOID COMPONENT OF THE EFFECT OF THE LACRIMAL GLANDS ON WOUND HEALING

UDC 617-001.4-003.9-02:617.
764.1-008.839.43-074

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KEY WORDS: lacrimal glands, sensitivity to pain, endorphins, structural homeostasis.

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We showed previously [2] that healing of skin wounds in rats and guinea pigs depends essentially on the functional state of the lacrimal glands. Activity of the lacrimal system increases significantly during exposure to painful stimulation [7], and it has accordingly been suggested that the lacrimal glands form part of a functional system which regulates nociceptive sensation and maintains structural homeostasis [3, 5]. Interaction between these two systems must evidently take place, because pain, as a signal of injury [9], must activate a complex of repair mechanisms, of which the lacrimal glands may be one component. The role of connecting link between them is evidently played by opioid peptides which, on the one hand, control sensivity to pain [11, 14] and, on the other hand, stimulate wound healing [4].

In order to reveal the opioid component in the activity of the lacrimal glands, it was decided to study the effect of their excitation and removal on sensitivity to pain, on endorphin levels in the blood and gland tissue, and also on wound healing under conditions of opioid receptor blockage.

EXPERIMENTAL METHOD

Experiments were carried out on 320 male Wistar albino rats weighing 180-220 g. The lacrimal glands were excited by four applications (with an interval of 3 days) of bilateral thermal stimulation of the conjunctiva by means of a thermocautery (60-70°C) under ether anesthesia. In the control animals the skin of the supraciliary arches was stimulated. Total surgical lacrimectomy (experiment), the operation of mock lacrimectomy (control), and removal of a dorsal skin flap were carried out under pentobarbital anesthesia (35 mg/kg, intraperitoneally). Sensitivity to pain was evaluated by the "tail-flick" method, by measuring the change in the temporal parameter of the pain threshold (PT). From six to eight measurements were made on each animal. To determine PT the animals were used only once in the experiment, to exclude any effect of adaptation of temperature receptors and the learning factor. The time course of wound healing was determined by planimetry and by calculation of the mean time of complete healing (MTCH). Naloxone (Sigma, USA), in a dose of 2 mg/kg, was injected intraperitoneally twice a day for 10 days. The plasma endorphin concentration was determined by the method in [1] and the concentration of immunoreactive α-, β-, and γ-endorphins in lacrimal gland extracts by the method in [8]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Excitation of the lacrimal apparatus led to changes in PT of the rats (Fig. 1). Five minutes after recovery from ether anesthesia, PT was 64% higher than in animals of the corresponding control group, but later it gradually returned to normal, and after 2 h it did not differ statistically significantly from the control. After 3 h, PT was significantly reduced to 74% of the control value.

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0007-4888/87/0012-1739$12.50

TABLE 1. Plasma Concentration of α- and γ-Endorphins (in fmoles/ml) of Rats in Response to Stimulation and Removal of the Lacrimal Glands (M ± m)

Time after procedure	Excitation of lacrimal glands		Removal of lacrimal glands	
	α-endorphin	γ-endorphin	α-endorphin	γ-endorphin
Control	93,8±6,42 (7)	34,9±2,68 (7)	106,6±13,5 (7)	34,1±1,9 (6)
10—15 min	108,1±12,2 (6)	26,3±1,02* (8)	—	—
3 h	129,0±6,02* (7)	34,2±1,08 (8)	—	—
24h	131,6±9,74* (6)	28,7±2,07 (6)	—	—
5 days	—	—	196,6±19,7* (7)	27,9±0,81* (7)

Legend. *p < 0.05 compared with control. Number of animals given in parentheses.

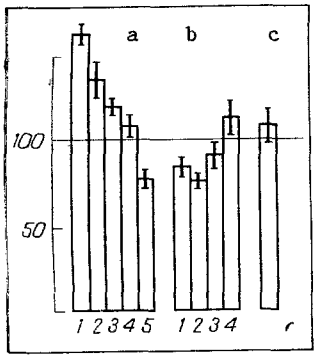

Fig. 1

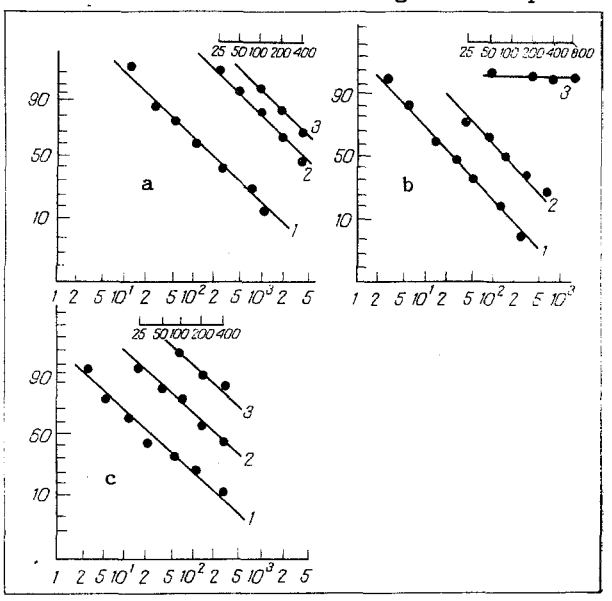

Fig. 2

Fig. 1. PT of rats depending on functional state of lacrimal glands (in % of control). a: 1-5) 5, 30, 60, 120, and 180 min, respectively, after stimulation of conjunctiva; b: 1-4) 4, 6, 10, and 35 days, respectively, after total bilateral lacrimectomy; c) in response to stimulation of conjunctiva in lacrimectomized rats on 5th day after operation, 5-10 min after recovery from anesthesia.

Fig. 2. Displacement of ^{125}I-endorphins from their complexes with specific antisera by α-endorphin (a, 1), β-endorphin (b, 1), and γ-endorphin (c, 1) and by extracts of lacrimal glands (2 – orbital, 3 – extraorbital parts). Abscissa, peptide concentration (in fmoles per sample), ordinate, $(B/B_0)\cdot 100$, where B_0 indicates binding of the ^{125}I-peptide with the corresponding antiserum in the absence of unlabeled peptide, B the same in the presence of synthetic peptide or extract. The scale on top of the graphs shows the quantity of extract (in μl per sample).

On the 4th day after total removal of the extraorbital, orbital, and palpebral portions of the lacrimal glands, i.e., the end of the period of acute inflammation and of the postoperative stress-induced change in sensitivity to pain, PT was 16% lower ($p < 0.05$) than in animals undergoing the mock lacrimectomy. PT remained low on the 6th day, when it averaged 74% ($p < 0.05$), but by the 10th day it reached the control value.

To study the role of the lacrimal glands in the changes observed in sensitivity to pain, the effect of conjunctival stimulation on PT was studied in lacrimectomized rats. The investigation was conducted 5 days after the operation, when the increase in excitability was most marked. From 5 to 10 min after recovery from the ether anesthesia, during which thermal stimulation of the conjunctiva was given, no change in PT was found: it varied within the limits of values recorded in lacrimectomized animals with thermal stimulation of the skin of the superciliary arches.

TABLE 2. Endorphin Concentrations (in fmoles/mg) in Tissues of Rat Lacrimal Glands (M ± m)

Tissue	α-Endorphin	β-Endorphin	γ-Endorphin
Lacrimal glands: orbital part	3,84±0,22	3,72±0,31	0,63±0,04
Extraorbital part	1,32±0,12	0,02±0,0	0,08±0,01
Hypothalamus	11,32±0,67	134,49±13,6	3,54±0,73
Cerebral cortex	1,17±0,15	1,53±0,41	0,30±0,14

Legend. Endorphin concentrations in cortex and hypothalamus given for comparison. Standard error reflects error of peptide radioimmunoassay in extracts of pooled lacrimal gland homogenates.

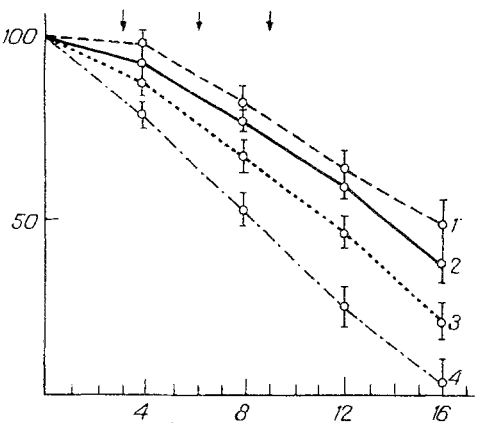

Fig. 3. Effect of excitation of lacrimal glands, injection of naloxone, and lacrimal gland stimulation coupled with injection of naloxone on time course of contraction of skin wounds in rats. Arrows indicate days of thermal stimulation of conjunctiva or skin or superciliary arches. Abscissa, time of investigation (in days); ordinate, average area of wound (in % of original area). 1) Injection of naloxone, 2) control, 3) excitation of lacrimal glands together with injection of naloxone, 4) excitation of lacrimal glands only.

These results demonstrate the role of the functional state of the lacrimal glands in regulation of the level of sensitivity to pain, for conjunctival stimulation in lacrimectomized animals did not affect PT. To determine the role of neuropeptides under these experimental conditions, plasma concentrations of α- and γ-endorphins were studied (Table 1).

Under the influence of excitation of the lacrimal glands, the plasma γ-endorphin concentration 10-15 min after recovery from the anesthetic was reduced on average by 24.7% ($p < 0.05$). However, its concentration returned to normal after 3 h. The α-endorphin level behaved differently. After 10 min its concentration began to rise, and reached 137.5% ($p < 0.05$) relative to the control after 3 h. At the end of 24 h its level still remained high (140%; $p < 0.01$).

The blood level of opioid peptides showed highly distinctive changes in lacrimectomized rate. On the 5th day after the operation, i.e., when the level of sensitivity to pain was highest, the γ-endorphin concentration was significantly reduced to 82%, and the α-endorphin level increased to 184% compared with the control.

Comparison of the trend of the rise in the blood α-endorphin concentration under the influence of excitation of the lacrimal apparatus with data on wound healing and changes in sensitivity to pain leads to the conclusion that opioid peptides may determine the analgesic and reparative effect, despite a very brief fall of the plasma γ-endorphin concentration. The increase in sensitivity to pain 3 h after stimulation of the conjunctiva, in assertion with a fairly high α-endorphin level, may perhaps be connected with the appearance of β-endorphin fragments with antagonistic properties [10, 12, 13]. In animals undergoing total lacrimectomy, the development of an endorphin deficiency in the blood was suggested, for under these conditions PT was lowered and repair processes inhibited. However, the opposite direction of the response of the endorphins indicates an important role not only of their absolute content, but also of their relative levels. Because of the absence of information on changes in blood concentrations of the other opioid peptides, no final conclusion can be drawn regarding the character of the relationship between lacrimal gland activity, the level of sensitivity to pain, and blood neuropeptide levels.

The study of the endorphin concentrations in the lacrimal gland tissues showed that it was particularly high in the orbital parts of the lacrimal apparatus (Table 2) and commensurate with their level in the cerebral cortex. An increasing quantity of extract, just as of synthetic endorphins, inhibits binding of the corresponding ^{125}I-peptide with the specific antiserum equally. The similar course of the competition curves (Fig. 2) indicates a close relationship between or complete identity of the competing substance of the lacrimal gland extracts and the synthetic peptide. The concentration of α- and γ-endorphins in the tissue of the extraorbital lacrimal glands was significantly lower, and no β-endorphin was found. This is in agreement with our own data showing the somewhat less important role of the extraorbital lacrimal glands in repair of skin wounds in rats. To determine the role of opioid peptides in regulation of repair processes by the lacrimal apparatus, we studied this phenomenon against the background of receptor blockade by naloxone.

It will be clear from Fig. 3 that bilateral thermal stimulation of the conjunctiva accelerated contraction of skin wounds considerably and significantly. MTCH for animals of this group was 17.5 ± 0.53 days, compared with 22.2 ± 0.84 days ($p < 0.05$) in control rats (with thermal stimulation of the skin of the superciliary arches). Injection of naloxone into rats with thermal stimulation of the skin of the frontal region inhibited contraction a little and lengthened MTCH, but not significantly (24.1 ± 0.78 days). Excitation of the lacrimal glands preceded by opioid receptor blockade by naloxone was accompanied by a significant decrease in the rate of wound healing at all times of investigation compared with animals not receiving naloxone. The area of the wounds on the 12th and 16th days was 23 and 21%, respectively, greater ($p < 0.05$). MTCH in this group was 21.9 ± 0.62 days ($p < 0.05$). However, complete blockade of the effect of excitation of the lacrimal glands on repair processes by naloxone at the contraction stage could not be obtained, although the dose chosen reliably inhibits transcranial electroanalgesia [6]. The failure of this attempt suggests the presence of another, nonopioid mechanism of the effect of the lacrimal glands on repair of injuries.

It can be concluded from analysis of the results that opioid peptides play an important role in the physiological function of the lacrimal apparatus studied in this investigation. This is shown by the presence of endorphins in the tissue of the lacrimal glands, correlation of the functional state of the glands with the level of sensitivity to pain and the blood endorphin concentrations, and partial blockade of the effect of the lacrimal glands on repair processes by naloxone. Endorphins evidently participate in the response of the organism to injury, by regulating PT, by exerting an antistressor action, and by helping to maintain structure homeostasis.

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