2. S. D. Varfolomeev, S. V. Zaitsev, and A. T. Mevkh, Progress in Science and Technology. Series: Bioorganic Chemistry [in Russian], Vol. 3, Moscow (1985).
3. F. I. Ershov and N. A. Askarkhodzhaev, Usp. Sovrem. Biol., 89, 394 (1980).
4. Ya. E. Khesin, A. M. Amchenkova, A. N. Narovlyanskii, et al., Vest. Akad. Med. Nauk SSSR, No. 1, 80 (1984).
5. J. E. Blalock and E. M. Smith, Proc. Natl. Acad. Sci. USA, 77, 5972 (1980).
6. J. E. Blalock and E. M. Smith, Biochem. Biophys. Res. Commun., 101, 472 (1981).
7. N. Dafny, Life Sci., 32, 302 (1983).
8. N. Dafny, Neurotoxicology, 4, 235 (1983).
9. P. Lengyel, Annu. Rev. Biochem., 51, 251 (1982).
10. C. Reyes-Vasquez, N. Weisbrodt, and N. Dafny, Life Sci., 35, 1015 (1984).
11. R. Simantov, S. R. Childers, and S. H. Snyder, Mol. Pharmacol., 47, 319 (1978).
12. S. H. Snyder, C. B. Pert, and G. W. Pasternak, Ann. Intern. Med., 81, 319 (1974).

OPIOID ACTIVITY OF PEPTIDES AND HEALING OF SKIN WOUNDS

V. A. Vinogradov, S. E. Spevak, K. N. Yarygin, N. V. Korobov, A. I. Solov'eva, A. B. Shekhter, and M. I. Titov

UDC 616.5-001.4-003.9-02:615.31:[547.95:547.943

KEY WORDS: opioid peptides; receptors; healing of skin wounds.

It has been shown experimentally and clinically in recent years [2-5] that certain peptides, which are enkephalin analogs, can stimulate repair of injuries to many tissues. At the same time, enkephalins are known to play an important role in the activity of peptidergic antinociceptive centers [8, 14]. The change in their functional activity arises under the influence of nociceptor stimulation [9], indicating a disturbance of the structural integrity of the tissues [6]. Hence it may be postulated that endogenous opioids not only regulate sensitivity to pain in vivo, but also are evidently involved in the system regulating structural homeostasis. Exogenous ligands, structural analogs of the enkephalins, may act in accordance with the same principle. Pharmacological investigations [1, 7] have shown that most of them have an analgesic action when injected intracisternally and systemically. However, to elucidate the mechanism of the effect of endogenous peptides and their structural analogs on the healing process, the first essential is to establish the link between opioid activity and regulation of repair processes.

The aim of this investigation was to study peripheral and central opioid activity of dalargin, its four analogs, and also of FK-33824, DADLE, met-enkephalin, morphine, and naloxone and to compare it with their effects on the healing of dorsal skin wounds in rats.

EXPERIMENTAL METHOD

Peripheral opioid activity of the peptides was assessed by their ability to inhibit contractions of an isolated segment of the ileum of noninbred male guinea pigs [11] and of the isolated vas deferens of noninbred mice [10], evoked by electrical stimulation. The preparations were placed in a constant-temperature cell 10 ml in volume, with modified Krebs' solution and stimulated by an electric current: the ileum — by pulses 1 sec in duration with a frequency of 0.1 Hz, the vas deferens by series of 4-6 pulses with a duration of 0.5 msec, an interval of 2 msec, and a frequency of 0.15 Hz. Contractions of the organs were recorded under isometric conditions by means of a K 30 transducer (Hugo Sachs Elektronik) on an MS 6601 automatic writer (Watanabe). The peptides were dissolved in distilled water and added to the surrounding solution in a volume of 5-50 μl. Activity of the peptides was expressed as the index EC_{50} — the concentration causing a reduction of 50% in the amplitude

Laboratory of Experimental Therapy, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 89-91, July, 1987. Original article submitted June 26, 1986.

TABLE 1. Opioid Activity and Effect of Peptides (M ± m)

Chemical structure of peptide	Peripheral receptors			Brain receptors		MTCH	
	Blocking activity EC_{50}, nM		EC_{50} A / EC_{50} B	IC_{50}, mM	K_i, nM	days	percent of control
	guinea pig ileum (A)	mouse vas deferens (B)					
1 Tyr-D-Ala-Gly-Phe-Leu-Arg dalargin	43,7±5,9 (22)	20,3±4,3 (4)	2,2	10	3,0	19,2±0,9 (10)	67*
2 Tyr-D-Ala-Gly-MePhe-Leu-Arg	36,2±8,6 (5)	203±33 (4)	0,18	11	2,6	21,2±1,8 (8)	73,8*
3 Tyr-D-Ala-Gly-Phe-Leu-D-Arg	19,0±6,0 (3)	37,3±10,0 (4)	0,51	13	3,0	25,4±0,8 (9)	88,5*
4 Tyr-Ala-Gly-Phe-Leu-Arg	$>5\cdot10^3$	$>10^4$	—	$>10^4$	—	27,5±1,5 (10)	95,8
5 Tyr-Ala-Gly-Phe-Gly-Arg	$>10^4$	$>10^4$	—	$>10^4$	—	30,3±1,2 (10)	105,5
6 Tyr-D-Ala-Gly-MePhe-Met-O—ol (FK-33824)	3,2±1,2 (11)	92,6±32 (7)	0,035	3,0	1,0	21,2±1,3 (10)	73,8*
7 Tyr-D-Ala-Gly-Phe-D-Leu (DADLE)	16,9±4,4 (5)	2,6±0,9 (9)	6,5	13	3,9	21,8±0,7 (10)	76,0*
8 Tyr-Gly-Gly-Phe- (met-enkephalin)	150±45 (5)	13,8±1,1 (6)	11,0	80	18,0	21,8±1,1 (10)	76,0*
Morphine	70±21 (5)	336,0±80 (5)	0,21	—	—	23,2±0,8 (10)	81,0*
Physiological saline	—	—	—	—	—	28,7±1,0 (10)	100

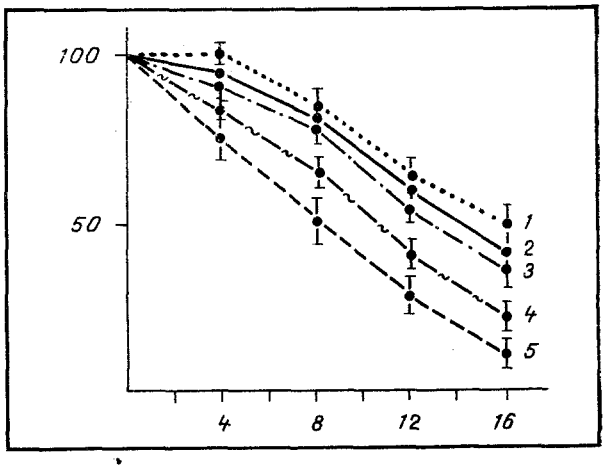

Fig. 1. Effect of naloxone (1), physiological saline (2), dalargin preceded by naloxone (3), morphine (4), and dalargin (5) on time course of contraction of skin wounds in rats. Abscissa, time after operation (in days); ordinate, change in area of wounds (in % of original, taken as 100).

of contraction of the organs to electrical stimulation. The ratio between mu-receptor activity (the guinea pig ileum) and delta-receptor activity (the mouse vas deferens) also was calculated so as to assess the degree of selectivity of the peptides for different types of receptors.

Central opioid activity of the peptides was studied by determining their binding with cell membrane receptors of the rat brain by the method in [13], with certain modifications. The incubation mixture, with a volume of 500 µl, contained the membrane fraction of the brain (150 µg as protein), 0.8-1.5 nM ^{3}H-naloxone, and $5\cdot10^{-5}$ bacitracin in buffer of the following composition: 5 mM HEPES, 120 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$, pH 7.4. Incubation was carried out for 1 h at 25°C in the absence and presence of different concentrations of the test substances and of naloxone. The samples were then filtered through GF/B filters, the filters were washed with 10 ml of buffer, and radioactivity bound with them was determined on a scintillation spectrometer. Specific binding of naloxone was calculated as the difference between total binding in the absence and presence of 2000 nM of unlabeled naloxone. The concentration (IC_{50}) at which specific binding of naloxone was inhibited by half, was determined for each substance tested. Using the Cheng–Prusoff equation: $K_i = \frac{IC_{50}}{1 + \frac{[L^*]}{K_D^*}}$

where [L*] stands for the concentration of labeled naloxone and K_D^* the dissociation constant of ^{3}H-naloxone and the receptor, the inhibition constant of naloxone binding with opioid receptors was calculated, and served as a measure of the affinity of the test peptide for opiate receptors of the rat brain.

The time course of healing of full-thickness skin wounds (diameter 17 mm) of the dorsal region in rats (127 male Wistar rats weighing 220-250 g) was assessed by planimetry, calculation of the mean times of complete healing (MTCH) on the basis of recording the time of separation of the secondary scab and complete epithelization of the wound defect, and also by histological and histochemical methods following treatment with dalargin. The histological material was taken from five experimental and five control animals 2, 4, 7, 10, 20, and 30 days after the operation. All the peptides, obtained from the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, were injected in a dose of 10 μg/kg (intraperitoneally) twice a day in physiological saline for 5 days. Naloxone was injected in a dose of 2 mg/kg and morphine in a dose equimolar to met-enkephalin, by the same scheme.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that dalargin was about equally effective on mu- and delta-opioid receptors. Dalargin also exhibited high activity toward brain receptors. The structural analog of dalargin with D-arginine in position 6 (No. 3) did not differ significantly from dalargin in its peripheral and central opioid activity. However, methylation of phenylalanine in position 4 (No. 2) led to a decline of delta-opioid activity toward peripheral receptors, but did not change binding with brain receptors. Replacement by L-alanine in position 2 (Nos. 4 and 5) deprived the peptide of its "opioid" properties. In this group of peptides all compounds with opioid activity stimulated wound healing considerably, whereas those without opioid activity (Nos. 4 and 5) had no such effect. Dalargin, which shortened MTCH of the wound by 33% ($p < 0.05$) compared with that in the group of control animals, receiving physiological saline, was particularly active.

The mu-receptor agonist FK 33824 and the delta-receptor agonists DADLE and met-enkephalin, besides their high opioid activity, also had a marked wound-healing action, shortening MTCH by 26.2, 24, and 24% respectively, which is comparable with the efficacy of the dalargin analogs with established opioid activity.

Thus, a stimulating effect on wound healing, among the series studied, is characteristic only of peptides with opioid activity, but it is unconnected with their effect on any particular type of peripheral opioid receptors. The same conclusion can be drawn from the results of the experiments with morphine and naloxone (Fig. 1). Morphine, in a dose equimolar to dalargin, accelerated wound contraction a little and shortened MTCH by 19%. These results are in agreement with the report [12] that morphine in a dose of 20 μg daily slowed the rate of natural cell death in the ciliary ganglion of avian embryos, and this effect was blocked by naloxone. Characteristically, higher doses of morphine had no such effect. The authors cited postulate a direct effect of opiates on preservation of cells of the autonomic nervous system. In the present experiment naloxone inhibited the course of wound contraction and increased MTCH, but not significantly. It completely blocked the wound-healing effect of dalargin. Some workers [15] also observed a change in size of the brain and in the number of cells in newborn rats under the influence of opioid receptor blockade by naltrexone. According to their opinion, opiate receptors and opioid peptides may be directly involved in the cellular events connected with growth, and may also serve as growth regulators.

Opioid peptides of petidergic antinociceptive centers, released by traumatic agents, and also opioids synthesized at the periphery, are thus evidently the humoral component in the maintenance of structural homeostasis and regulation of repair processes in vivo.

LITERATURE CITED

1. Zh. D. Bespalova, N. V. Korobov, M. I. Titov, et al., Farmakol. Toksikol., 45, No. 2, 39 (1982).
2. V. A. Vinogradov and V. M. Polonskii, Byull. Éksp. Biol. Med., No. 5, 548 (1985).
3. O. B. Il'inskii, S. E. Spevak, A. I. Solov'eva, et al., Neuropeptides: Their Role in Physiology and Pathology [in Russian], Tomsk (1985), p. 58.
4. V. M. Polonskii and V. A. Tishchenko, Neuropeptides: Their Role in Physiology and Pathology [in Russian], Tomsk (1985), p. 147.

5. V. D. Slepushkin, V. L. Pavlenko, Yu. B. Lishmanov, et al., Epidemiology, Diagnosis, Treatment, and Rehabilitation of Cardiovascular Diseases [in Russian], Kaunas (1984), p. 514.
6. V. M. Khayutin, Abstract of Proceedings of a Scientific Session of Pain Mechanisms and Analgesia. Department of Medico-biological Sciences, Academy of Medical Sciences of the USSR [in Russian], Moscow (1979), p. 17.
7. O. N. Chichenkov and N. V. Korobov, Farmakol. Toksikol., 47, No. 1, 23 (1984).
8. J. E. Adams, Pain, 1, 161 (1976).
9. G. K. Aghajanian, R. J. Wang, and J. Baraban, Brain Res., 153, 169 (1978).
10. J. Hughes, H. W. Kosterlitz, and F. M. Leslie, Br. J. Pharmacol., 53, 371 (1975).
11. H. W. Kosterlitz, R. J. Lydon, and A. J. Watt, Br. J. Pharmacol., 39, 398 (1970).
12. S. D. Meriney, D. B. Gray, and G. Pilar, Science, 228, 1451 (1985).
13. R. Simantov, S. R. Childers, and S. H. Snider, Mol. Pharmacol., 14, 69 (1978).
14. L. Terenius, Second World Congress on Pain, Washington (1978), p. 171.
15. I. S. Zagon and P. J. McLaughlin, Science, 221, 1179 (1984).

EFFECT OF ANTIGENIC STIMULATION ON PROLIFERATIVE ACTIVITY OF HEMATOPOIETIC STEM CELLS IN SPLENECTOMIZED MICE

K. V. Gaidul', I. G. Tsyrlova, and V. A. Kozlov

UDC 612.017.1:612.119

KEY WORDS: hematopoietic stem cell; splenectomy; immune spleen cells.

The solution to the problem of the mechanisms of regulation of proliferation and differentiation of the bone marrow hematopoietic stem cell (BMC) during antigenic stimulation depends on the elucidation of the role of the peripheral lymphoid organs and their cells in these processes. We know that the spleen plays an important role in the development of the immune response to thymus-dependent (SRBC — sheep's red blood cells) and thymus-independent (PPS — pneumococcal polysaccharide SS 111) antigens [4, 9]. Meanwhile it has been shown that after injection of another thymus-independent antigen — *E. coli* lipopolysaccharide (LPS) into mice, a marked immune response to this antigen is formed even in the absence of the spleen [11]. Immunization of mice with SRBC, PPS, and LPS also caused stimulation of proliferation of hematopoietic stem BMC [3, 6].

Since the role of the hematopoietic stem cell (CFUs — colony-forming unit of the spleen) in immunopoiesis is no longer disputed [3], it was considered important to determine the proliferative activity of CFUs in splenectomized mice in response to injection of various antigens.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6)F_1 mice weighing 18-20 g, obtained from the Svetlye Gory nursery, Academy of Medical Sciences of the USSR. Antigenic stimulation consisted of intraperitoneal injection of SRBC in a dose of $2 \cdot 10^8$, PPS, generously presented by P. J. Baker (USA), in a dose of 100 μg, and *E. coli* LPS (from Difco, USA) in a dose of 100 μg. Immunization was carried out 72 h before determination of proliferative activity of CFUs in the bone marrow of splenectomized mice and mice undergoing a mock operation, by the method in [5]. The recipients (20-25 mice) of each group were given an intravenous injection of BMC from three to five donors. The results of two or three experiments are pooled in Tables 1 and 2. Splenectomy was performed under ether anesthesia 45 days before the experiment. Immune spleen cells (ISC) were obtained 48 h after intravenous immunization of normal animals with SRBC in a dose of $2 \cdot 10^8$. Cell populations of ISC, enriched with macrophages and lymphocytes, were obtained by the method in [10].

Institute of Clinical Immunology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. I. Borodin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, 91-93, July, 1987. Original article submitted June 26, 1986.

 0007-4888/87/0007-0988$12.50