ANALGESIC EFFECT OF VARIOUS FRACTIONS OF BONE MARROW CELL CULTURE SUPERNATANT IN PATHOLOGICAL PAIN

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UDC 616.8-009.7-092:612.884.064:612.419.015.2

KEY WORDS: fractions of bone marrow cell culture supernatant; pain syndrome of spinal origin; generator of pathologically enhanced excitation.

A group of bioregulatory peptides known as myelopeptides (MP), which possess functional heterogeneity, is synthesized in human and animal bone marrow. MP have an immunostimulating effect on various types of immunocompetent cells [5, 6, 8, 10], and they also have an analgesic effect, which is realized through opioid receptors [1, 4, 7]. The analgesic action of MP is manifested against both physiological and pathological pain, namely the pain syndrome induced by creation of a generator of pathologically enhanced excitation (GPEE) [3] in the dorsal horns of the spinal cord [2]. On the basis of MP with mol. wt. of about 2.0 kilodaltons, which possess the strongest immunoregulatory properties, a new immunocorrective preparation has been developed, known as myelopide, which has an analgesic effect in man [9].

The aim of this investigation was to evaluate the analgesic action of substances which are components of different fractions of cultures of bone marrow cells and to compare their analgesic action with that of myelopide.

EXPERIMENTAL METHOD

MP were isolated from the supernatant of cultures of hog bone marrow cells, concentrated tenfold, by gel-filtration on Sephadex G-25 (fine), equilibrated with physiological saline (pH 7.2). The fractions thus obtained were concentrated tenfold and used in the experiments. Three fractions were obtained: 1) with mol. wt. > 2.0 kD, 2) (myelopide) with mol. wt. of 2.0 kD, and 3) with mol. wt. of < 1.0 kD. Experiments were carried out on noninbred male rats weighing 200-220 g. A model of pathological pain was produced by creating a GPEE in the dorsal horns of lumbosacral segments of the spinal cord with the aid of penicillin [2]. Penicillin was applied to the dorsal surface of the lumbosacral segments in an agar wafer (10 × 4 × 1.5 mm) on the left side. To 1 ml of 1% agar 1500 U of penicillin was added. Development of the pain syndrome was assessed on a 3 point scale. Fractions were injected intraperitoneally in doses of 1-10 mg protein/kg body weight, determined by Lowry's method, in a volume of 0.3-0.5 ml. In individual experiments the supernatant of bone marrow cell cultures, concentrated tenfold, was injected intraperitoneally in a volume of 0.5-1.0 ml. Animals of the control group received an injection of the same volume of physiological saline.

EXPERIMENTAL RESULTS

The analgesic effect of individual fractions relative to this pain syndrome of spinal origin described above is illustrated in Figs. 1 and 2. Different fractions of supernatant of bone marrow cell cultures were injected at the height of development of the pain syndrome, which lasted about 3.5 h (Fig. 1, I). It will be clear from Figs. 1 and 2 that after injection of all the test fractions a marked dose-dependent analgesic effect developed. Fraction 1 in a dose of 1.0 mg/kg caused virtually complete abolition of the pain syndrome 40-50 min after injection (Fig. 2, II, a). The action of the fraction persisted throughout the period of observation (3-3.5 h). An increase in the dose of fraction 1 to 2.5-10.0 mg/kg body weight led to reduction of its analgesic action, and individual features of the pain syndrome persisted up to a level of 1.0-1.5 points (Fig. 2, II, b, c, d).

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR. Institute of Immunology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 653-655, December, 1988. Original article submitted June 15, 1988.

 0007-4888/88/0012-1676$12.50

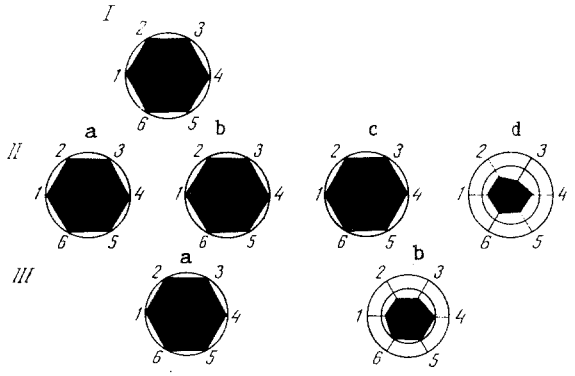

Fig. 1

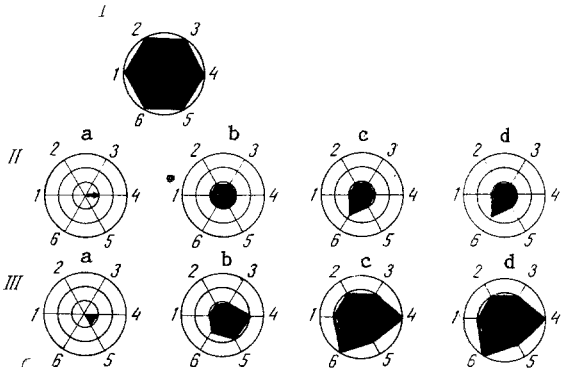

Fig. 2

Fig. 1. Effect of myelopide (fraction 2) and whole supernatant of bone marrow cell cultures on individual components of pain syndrome of spinal origin. I) Initial background (peak manifestation of pain syndrome); II) effect of myelopide (fraction 2) injected intraperitoneally in a dose of: a) 1.0 mg/kg, b) 2.5 mg/kg, c) 5.0 mg/kg, and d) 10.0 mg/kg body weight; III) effect of whole supernatant injected intraperitoneally in a dose of: a) 0.5 ml, b) 1.0 ml per animal. Features of pain syndrome recorded: 1) frequency of spontaneous attacks, 2) duration of one attack, 3) intervals between attacks, 4) response to provocation, 5) vocalization, 6) generalized motor reaction (shown graphically in the form of vectors). Here and in Fig. 2: estimate of severity of individual features of pain syndrome on a 3 point scale, each circle representing 1 point, counting from the center: 0) all features absent, 1) mild degree, 2) average degree, 3) severe degree of its manifestation.

Fig. 2. Effects of individual fractions of supernatant of bone marrow cell cultures on individual components of pain syndrome of spinal origin. I) Initial background (peak manifestation of pain syndrome); II) effect of fraction 1; III) effect of fraction 3 of supernatant, injected intraperitoneally in a dose of: a) 1.0 mg/kg, b) 2.5 mg/kg, c) 5.0 mg/kg, d) 10.0 mg/kg. Features of pain syndrome recorded: 1) frequency of spontaneous attacks, 2) duration of one attack, 3) intervals between attacks, 4) response to provocation, 5) vocalization, 6) generalized motor response (represented by vectors). Severity of individual features of pain syndrome assessed on a 3-point scale; each circle represents 1 point, counting from the center: 0) all features absent, 1) mild degree, 2) average degree, 3) severe degree.

Similar effects were produced by injection of fraction 3 into the rats (Fig. 2, III, a-d). The maximal analgesic effect under the action of fraction 3 developed with a dose of 1.0 mg/kg. Under these circumstances the pain syndrome was reduced to 0.5 point (Fig. 2, III, a). Doses of 2.5-10.0 mg/kg caused a decrease of analgesia to 2.0-2.5 points (Fig. 2, III, a). Doses of 2.5-10.0 mg/kg caused a reduction of analgesia to 2.0-2.5 points (Fig. 2, III, b, c, d). Incidentally, the ability of fractions 1 and 3 of the supernatant of the bone marrow cell cultures to give their greatest analgesic effect when their dose was reduced distinguishes their action in principle from that of the known narcotic analgesics, for which the effect is directly dependent on dose.

Fraction 2 (myelopide) and also the whole supernatant differed in their effectiveness from fractions 1 and 3. Potentiation of the analgesic action of myelopide was observed with an increase in dose. The maximal effect of myelopide developed when given in a dose of 10.0 mg/kg (Fig. 1, II, d). A further increase in the dose led to potentiation of the effect [4]. A direct dose-dependent effect also was characteristic of the whole supernatant of the bone marrow cell cultures after injection into the animals: When concentrated tenfold and injected in a volume of 0.5-1.0 ml per animal intraperitoneally, the supernatant reduced the intensity of the pain syndrome to 1.5 points (Fig. 1, III). Differences in the manifestation of the effects of the whole supernatant and of fractions 1 and 3 may be caused in various ways. Biologically active substances inducing analgesia are evidently present in whole supernatant in smaller amounts, and their relative proportions differ from those in fractions 1 and 3, isolated and concentrated separately. Furthermore, amino acids of the culture medium, present in quite high concentrations, may also have some effect on realization of the analgesic effect of the whole supernatant.

Thus bone marrow cells produce a group of substances with a wide range of molecular weight (0.3-150.0 kD) possessing a marked analgesic action and depressing a severe pain syndrome of spinal origin induced by a GPEE in the dorsal horns of the lumbosacral segments of the spinal cord. The possibility cannot be ruled out that high-molecular-weight substances contained in fraction 1 and possessing analgesic properties are precursors of low-molecular-weight bone marrow peptides. It is an interesting fact that, unlike the commercial preparation myelopide, medullary peptides with both higher and lower molecular weight give an analgesic effect in much lower concentrations. Another important property of the myelopeptides is the absence of muscle-relaxing and narcotic effects in the realization of their analgesic effect. These properties distinguish myelopeptides in principle from known narcotic analgesics of the morphine type.

The facts described above offer prospects of the creation of effective and harmless analgesics, and also of preparations with combined immunostimulating and analgesic action, on the basis of bone marrow peptide molecules.

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ABOLISHING STRESS-INDUCED ACTIVATION OF MYOCARDIAL REPARATIVE DNA SYNTHESIS BY AN INCREASED LOAD ON THE HEART

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UDC 616.127-008.939.633.2-02:613.863/-07:616.127-008.939.633.2-008.66-02:616.132-089.814

KEY WORDS: myocardium; stress; replication; DNA.

Under the influence of emotional—painful stress (EPS) damage to DNA and a subsequent increase in the rate of its reparative synthesis are observed in the heart cells [4]. Meanwhile the rate of replication rises in the heart cell nuclei [6]. As a result of compensatory hyperfunction of the heart (CHH) caused by coarctation of the aorta, the replication rate also is increased [13], but reparative DNA synthesis under these circumstances has not been studied. Moreover, it is not clear to what extent the rate of DNA synthesis during CHH in the heart cells depends on the increase in their function, and to what extent on operative stress, which inevitably arises in surgically created coarctation of the aorta and in other models of CHH.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 655-657, December, 1988. Original article submitted October 9, 1987.

0007-4888/88/0012-1678$12.50