Involvement of Opioid Receptors in the Production of Nonspecific Protective Responses

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The opioid system is known to play a role in determining the body's resistance to various destabilizing factors. The actions of many of these are mediated by interleukin 1 (IL-1), which also initiates a number of protective reactions [11]. Identifying the nature of the changes and the interactions of processes induced by this leukopeptide in relation to the opioid system's activity may provide an experimental basis for research to develop optimal methods for correcting the body's compromised resistance to such factors.

It has been established that IL-1 influences the synthesis and processing of proopiomelanocortin and the secretion of β-endorphin [10,16]. A recombinant IL-1 preparation has been shown to exert an analgesic effect and to have predominantly a central (naloxone-independent) mechanism of action in mice [13]. In a study using a radioreceptor method to examine opiate receptors of the guinea pig brain, IL-1 was found to interact with opiate receptors of the μ-type [9]. Our previous experiments on mice indicated that dalargin, a synthetic analog of leu-enkephalin, can prevent the bacterial lipopolysaccharide pyrogenal from enhancing the synthesis of C-reactive protein (CRP) [5].

The purpose of the present study was to examine the mechanisms by which native IL-1 interacts with dalargin during the production of an acute-phase reaction and to identify the possible analgesic effect of this interleukin.

MATERIALS AND METHODS

A partially purified preparation of native rabbit IL-1 was used (mol. wt. 17 kD; minimal pyrogenic dose 7 μg/kg body weight; maximal body temperature rise 1.56°C after intravenous injection; specific comitogenic activity 1.0×10^5 units/mg). Although

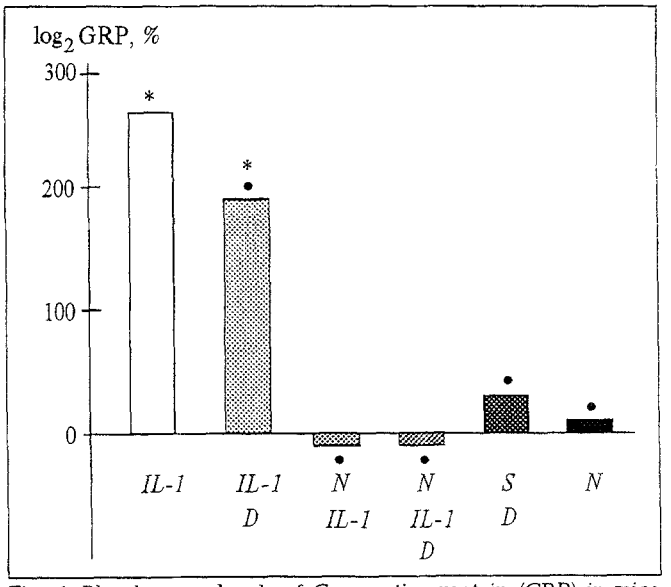

Fig. 1. Blood serum levels of C-reactive protein (CRP) in mice 48 h after the injection of IL-1, dalargin (D), and naloxone (N). The CRP level in mice injected with saline (S) alone was taken as 100%. The asterisks denote a significant difference from the mice injected with S and the dots, a significant difference from those injected with IL-1.

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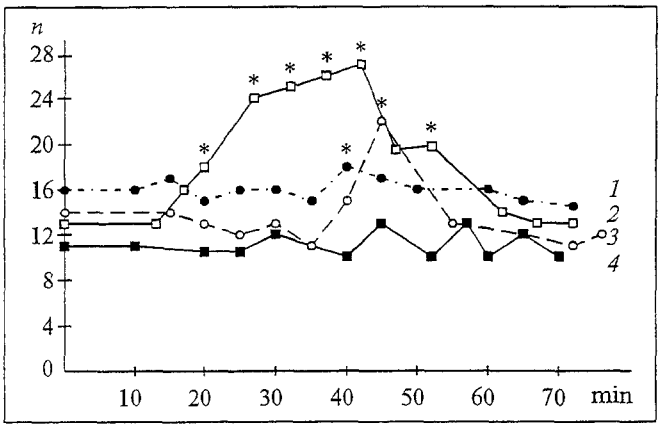

Fig. 2. Effect of IL−1 on the summation of nociceptive impulses (*n*) in the central nervous system of rabbits. The arrow indicates the injection of IL−1 and saline (S). Figures in parentheses are the numbers of rabbits per group. *1*) heated IL−1 (6); *2*) IL−1 (11); *3*) IL−1 + naloxone (10); *4*) S (7). The asterisks denote a significant difference from rabbits injected with S and the dots, a significant difference from the results before the injections.

the presence of minor impurities, such as tumor necrosis factor, IL-6, or other monokines, could not be ruled out in the partially purified preparation, the latter could be regarded as native rabbit IL-1 (referred to as IL-1 below), as was indicated by its biochemical assay [4], its high biological activity, and a comparison of its properties with those of the recombinant IL-1 preparation. We also used in this study the synthetic leu-enkephalin analog dalargin (produced by the Institute of Experimental Cardiology, Moscow); a diagnostic serum to CRP (from the Research Institute of Vaccines and Sera, St. Petersburg); a 0.1% solution of naloxone (Sigma, USA), which was injected 15 min before the injection of other preparations; and an apyrogenic physiological saline.

The first series of tests was carried out on 194 male hybrid CBA mice weighing 20-25 g. In the mice of each group, blood serum CRP was determined by an immunological technique [3] 48 h after the injection of the test preparations.

In the second series, performed on 34 male chinchilla rabbits 3100-3400 g in weight, IL-1 was studied for its possible analgesic effect following the injection of its pyrogenic dose using a model in which nociceptive impulses were summated in nonrigidly fixed rabbits [1]. Stimulation was carried out with subthreshold pulses via subcutaneous electrodes placed in the area of the posterior surface of the femur (stimulation parameters: frequency 1 Hz; pulse duration 1 msec; voltage 7-12 V, depending on individual responses of the animals). After the application of 10-12 pulses, the rabbit exhibited a general motor response. During the test, body temperature was measured with a rectal sensor and electrothermometer. The substances and their doses used in the mice and rabbits are indicated in Tables 1 and 2, respectively.

RESULTS

The results of tests with mice are summarized in Fig. 1. After the injection of dalargin together with IL-1, serum CRP levels were 1.5-fold lower than after the injection of IL-1 alone, although they were high in comparison with those in the control group, which received only saline. Preinjecting the mice with naloxone prevented the IL-1 from elevating the serum level of CRP.

IL-1 and IL-6 have been shown capable of inducing the synthesis of acute-phase proteins in the body, both by activating the central structures that regulate the blood level of these proteins and by stimulating the peripheral mechanisms of their synthesis, in particular by the hepatocytes [7,14]. Considering the administration route and dose of dalargin used in our tests, it is likely that this compound reduced CRP synthesis by acting, in the main, through the peripheral mechanisms regulating CRP production by the hepatocytes. Since the universal blocker of opioid receptors naloxone, preinjected with physiological saline or dalargin before IL-1, was found to prevent the latter from

TABLE 1. Administration of IL−1, Dalargin (D), Naloxone (N), Saline (S), and Their Combinations to Mice

Group	*n*	Substance, dose per mouse, and administration route
I	17	Intact mice
II	20	0.5 ml S i.m. and 0.2 ml S i.p.
III	47	50 μg IL−1 in 0.5 ml S i.m. and 0.2 ml S i.p.
IV	34	3.0 μg D in 0.2 ml S i.p. and 0.5 ml S i.m.
V	6	10 μg N s.c. and 0.5 ml S i.m.
VI	24	50 μg IL−1 in 0.5 ml S i.m. and 3 μg D in 0.2 ml S i.p.
VII	31	50 μg IL−1 in 0.5 ml S i.m. and 10 μg N s.c.
VIII	15	50 μg IL−1 in 0.5 ml S i.m., 3.0 μg D in 0.2 S i.p., and 10 μg N s.c.

Note. Here and in Table 2: *n* = number of animals; i.m. = intramuscularly; i.p. = intraperitoneally; s.c. = subcutaneously.

TABLE 2. Administration of IL-1, Naloxone (N), Saline (S), and Their Combinations to Rabbits

Group	n	Substance, dose per kg body weight, and administration route
I	11	7 μg IL-1 i.v. in 1.0 ml S
II	6	7 μg heated IL-1 i.v. in 1.0 ml S
III	10	7 μg IL-1 i.v. in 1.0 ml S and 0.5 mg N s.c.
IV	7	1.0 ml S i.v.

inducing a rise in the CRP level, both IL-1 and dalargin appear to have affected this process by engaging the opioid receptors.

Taking into account what is known about the allosteric effects of IL-1 with respect to ligands for receptors of the μ, Δ, and ε types [15] and about the central effects of IL-1 (including its analgesic effect [12,13]), as well as the structural similarly of a number of receptors for ligands of a peptide nature such as ACTH, endorphins, IL-1, and IL-2 [8], adopting the view that close ligand-receptor and receptor-receptor interactions occur between structurally similar ligands and receptors [2,6] may be of considerable help in understanding many of the physiological effects exerted by regulatory peptides.

Of interest in this context are the results of the tests with the rabbits (Figs. 2 and 3). In these tests, the number of subthreshold pulses required to produce a reflex response was increased, on average, twofold 13 min after the injection of IL-1 and during the subsequent 35 min. In rabbits preinjected with naloxone, this effect was virtually unobservable throughout the period during which naloxone was active in the animals (Fig. 2). In addition, IL-1 also prevented the development of fixation-induced hypothermia (unlike in rabbits injected with inactivated IL-1 or with saline), while preinjecting the rabbits with naloxone abolished the pyrogenic action of IL-1 (Fig. 3).

Thus, this study has revealed new properties in IL-1, namely its ability to delay the naloxone-dependent summation of peripheral nociceptive impulses in the central nervous system and to prevent the development of fixation hypothermia which can be abolished by naloxone. It should be noted that the summation of impulses was delayed during the period when the thermoregulatory reactions of the vascular and muscular systems were activated in rabbits, namely between the 13th and 45th minutes after the intravenous injection of a pyrogenic IL-1 dose; this possibly reflects a multifunctional role of interleukins in the early stages of acute-phase and inflammatory reactions. The model we used, in which nociceptive stimulation is a major, though probably not the only, determinant of the observed changes, enables us to interpret (with a degree of caution) the discovered effect of IL-1 as analgesic.

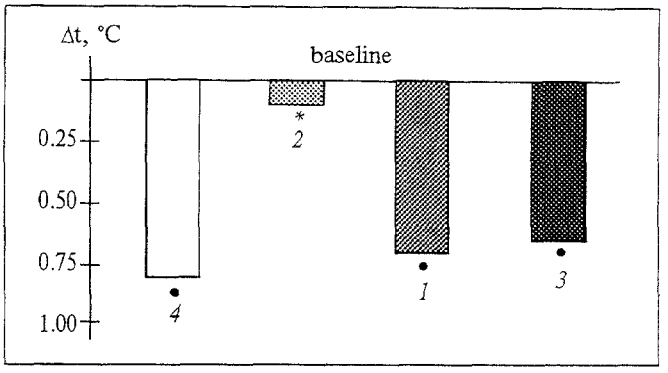

Fig. 3. Effect of IL-2 on the level of fixation-induced hypothermia in rabbits. Same designations as in Fig. 1.

The finding that IL-1 and dalargin act by a common mechanism on the receptors of CRP-synthesizing cells is a reflection of the close interrelationship between two components of the system regulating the production and course of an inflammatory acute-phase reaction - namely between biologically active substances (including interleukins) which initiate the changes involved, and opioid peptides which exert anti-inflammatory effects. Interaction at the level of cell receptors (for interleukins and opioids) appears to be one of the common pathways for the formation of nonspecific protective (acute-phase) reactions in the body, including the hypothermic, inflammatory, and antinociceptive reactions.

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Effect of Activation of the Mononuclear Phagocyte System on Binding of Low- and High-Density Lipoproteins to Rat Hepatocytes, Kupffer and Endothelial Cells

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The liver is the main organ regulating the lipoprotein (LP) and cholesterol (CH) blood levels. Low-density lipoproteins (LDL) are believed to transport CH to the peripheral tissues, whereas high-density lipoproteins (HDL) are responsible for reverse CH transport to the liver, where it is converted to bile acids [6]. Specific receptors to LDL and HDL are found on both hepatocytes and nonparenchymal elements, namely, endothelial and Kupffer cells [15]. Kupffer cells are a component of the mononuclear phagocyte system (MPS) and account for more then 20% of the total pool of macrophages in the organism [10]. Macrophages have been recently established to secrete a broad spectrum of cytokines, which induce various functional changes in different types of cells. Their involvement in the regulation of erythropoiesis, the immune response, and the proliferation of fibroblasts and vascular endothelium has also been shown [9]. At the same time, the role of macrophages in the regulation of lipid metabolism is poorly understood [4]. Previously we showed that stimulation of the MPS with lipopolysaccharides (LPS) leads to marked induction of protein synthesis [1] and activation of key enzymes of carbihydrate metabolism in hepatocytes [3]. The present study aimed to investigate the role of the MPS in regulating the LP receptors in different types of liver cells.

MATERIALS AND METHODS

Experiments were carried out on female Wistar rats weighing 180-200 g. Stimulation of the MPS was effected by injecting intravenously the bacterial LPS prodigiosane (Moskhimfarmpreparat, Russia) in a dose of 0.25 mg/kg. The animals were decapitated under light ether anesthesia after 24 and 72 hours. The liver cells were isolated by enzymatic digestion [2,15] using recirculatory perfusion of the liver with 0.03% collagenase (Boehringer Mannheim, Germany). Hepatocytes and nonparen-

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