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Morphine-induced changes in the activity of proopiomelanocortin and prodynorphin systems in zymosan-induced peritonitis in mice

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Abstract

We have shown that supplementation of proinflammatory agent with a high dose of morphine not only abolishes inflammation-related pain symptoms but also inhibits influx of leukocytes to the inflamed peritoneal cavity. Present investigations focused on effects of morphine on proopiomelanocortin and prodynorphin systems during zymosan-induced peritonitis. Males of SWISS mice were ip injected with zymosan (Z, 40 mg/kg) or zymosan with morphine (ZM, 20 mg/kg). At time 0 (controls) and 4 and 24 h after stimulation, peritoneal leukocytes (PTLs) were counted, PTL levels of opioid peptides (β-endorphin and dynorphin) measured by radioimmunoassays, while mRNAs coding their respective precursors (POMC and PDYN) and receptors (MOR and KOR) determined by QRT-PCR. Influx of inflammatory PTLs, mainly PMNs, was significantly delayed by morphine co-injection. Total levels of β-endorphin and dynorphin corresponded with PTL numbers, while levels per cell were similar in all groups except of β-endorphin, decreased in ZM at 4 h. Levels of both peptides in peritoneal fluid were increased in Z and ZM groups at 4 h, while at 24 h only in case of β-endorphin in Z group. POMC was increased only in ZM group at 4 h of peritonitis, while PDYN in both Z and ZM groups at the same time. MOR mRNA was increased 24 h after injection in Z and ZM groups, while KOR mRNA was similar in all groups except of decrease in Z at 24 h. In conclusion, endogenous opioids and their receptors are involved in zymosan-induced peritonitis and affected in various ways by morphine co-injection.
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Keywords: Peritonitis; Morphine; Proopiomelanocortin; Prodynorphin; Zymosan; Peritoneal leukocytes; PMN

1. Introduction

Administration of opioids in both humans and animal models results in significant alterations in immune system responsiveness [1]. Although the majority of studies have focused on phenotypic changes in immune cells after short- and long-term morphine administration, few studies have determined whether alterations in gene expression of endogenous opioids and their receptors accompany these effects. Endogenous opioid peptides are synthesized and processed within various types of immune cells at the site of inflammation [2–5]. The results of various studies indicate that endogenous opioids (primarily β-endorphin) released during stressful stimuli can interact with peripheral opioid receptors to inhibit nociception in inflamed tissue of rats [2,3,6]. It was also reported that inhibitory effect of μ- and δ-opioid receptor agonists in the gut is enhanced in intestinal inflammation. The effect is probably related to the increased number of functional opioid receptors [7,8].

We wish to draw attention to another model for investigations of the participation of opioid systems in inflammatory processes, namely to experimental peritonitis induced by i.p. injection of a sterile stimulant (e.g. zymosan). The conve-

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nience of this model relies on the possibility of a precise quantification of inflammation-related cells and soluble factors in samples of peritoneal fluid [9,10]. We have shown that supplementation of a stimulant with morphine significantly reduces the levels of chemotactic factors and inflammatory leukocytes in mice and fish [11], but not in frogs and toads [12] with experimental peritonitis. This corresponds with inhibited migration of mice and fish (but not frogs) morphine-treated leukocytes to zymosan-activated serum [13]. The resistance of frogs and toads to exogenous morphine may be connected with the presence of unique amphibian-specific endogenous opioids, dermorphins and deltorphins [14].

In mice, early stages of zymosan-induced peritonitis are accompanied by characteristic body writhes (consisting in a contraction of the abdominal muscles together with a stretching of hind limbs) considered to be pain symptoms [15]. Morphine supplementation to an irritant completely eliminates the inflammation-related pain symptoms already at the low doses (5 or 10 mg kg b.w.) while at the high dose (20 mg kg b.w.) additionally inhibits intraperitoneal influx of leukocytes in four out of five investigated strains of mice, including SWISS [16]. Therefore, local morphine administration might offer double profits during planned surgeries, being both antinociceptive and antiinflammatory [17]. Such a morphine-dependent modification of the inflammatory process would seem to be advantageous for the host, since inflammation-related cells and molecules, e.g. complement components [18], prostaglandins [19], and mast cell-derived mediators [20], are double-edged swords and their high concentration and/or prolonged action might be detrimental.

Recently we recorded expression of proenkephalin mRNA in exudatory leukocytes from the peritoneal cavity of mice with zymosan-induced inflammation [21]. This implies that these cells participate in the production of anti-nociceptive enkephalins accumulating in the inflamed peritoneal cavity [22]. We may speculate that morphine can support and/or replace the anti-nociceptive action of endogenous opioids released by leukocytes in the focus of inflammation and, as a consequence, an influx/accumulation of new leukocytes may be limited. Therefore, it is worth to study the effects of exogenous morphine on systems of endogenous opioids during inflammatory processes.

In our previous study on SWISS mice it turned out that the level of PENK mRNA which derived from peritoneal leukocytes was increased 4, 8 and 24 h after zymosan administration [21]. The above increase correlates with the absence of pain-related behaviours [16,17], what suggest an important role of this source of PENK-derived peptides in visceral pain. Besides PENK two other opioid systems, proopiomelanocortin (POMC) and dynorphin could be regarded as modulators of inflammatory states. Therefore, the aim of the present investigation was to evaluate whether these systems are involved in zymosan-induced peritoneal inflammation. Moreover, the goal of the study was also the question if morphine co-injection influences the activity of proopiomelanocortin and prodynorphin in cells which infiltrate focus of inflammation during peritonitis.

2. Materials and methods

2.1. Animals

Male SWISS mice (6–8 weeks age) weighing 25–32 g, purchased from the commercial supplier (Breeding of Laboratory Animals, Collegium Medicum, Krakow, Poland), were kept in a room with controlled temperature (22 °C) and lighting (lights on 8:00–20:00 h) and free access to food and water. The study protocol was approved by the local Committee of Animal Use and Care of the Institution in accordance with the International Association for the Study of Pain guidelines on ethical standards for investigations in animals (license no. 16/OP/2001). All experiments were conducted between 10:00 a.m. and 2:00 p.m. and were performed in triplicates in order to check reproducibility and to ensure accurate results

2.2. Schedule of drug administration and experimental design

Animals, four to six per group, handled before the experiment were either left untreated (0 time) or injected i.p. (the mouse was routinely held by the neck and the tail by the experienced investigator, and quickly aseptically injected in the ethanol-sterilised bottom left quadrant of the belly) with freshly prepared zymosan (Z groups) (2 mg/ml, 0.5 ml/25 g b.w.) (Zymosan A, Sigma, St. Louis, MO, USA) in sterile PBS, or with zymosan supplemented with morphine hydrochloride (20 mg/kg b.w.; ZM groups, Polfa, Kutno, Poland). The main aim of the present study was to compare the opioid system during inflammation induced by a single injection of zymosan only with that induced by zymosan supplemented with morphine, i.e. between Z and ZM groups of Swiss mice. Both of them were compared to the intact controls. To limit the number of animals and to restrict the complexity of experimental protocol and data analysis, the additional control groups injected with PBS were at present omitted. Moreover, in a series of our previous experiments the intraperitoneal puncture in mice handled before the experiment did not change the level of mRNA coding for opioid receptors. The results of such sham group were the same as in naïve animals (unpublished data). Therefore, the changes in opioid systems in groups injected with Z and ZM were compared only with naïve mice. Animals were decapitated at time 0 (controls) or 4 and 24 h after Z or ZM administration. Their peritoneal cavities were lavaged with 1 ml of PBS. Turk stained peritoneal leukocytes (PTLs), among them polymorphonoclear leukocytes (PMNs) and mononuclear cells (Mo) were counted in haemocytometer. Peritoneal exudate was centrifuged (15 min, 1500 rpm) and supernatants were stored at −20 °C for opioid peptides detection. Cell pellets, either total or adjusted to the constant number of PTL per vol-

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