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The influence of opioids on the humoral and cell-mediated immune responses in mice. The role of macrophages

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Abstract:
Background: Our experiments were aimed to test the influence of treatment with different opioids (morphine, fentanyl, methadone) on the humoral and cell-mediated immune responses.
Methods: Mice were treated intraperitoneally (*ip*) with opioids for several days and next either immunized with sheep red blood cells (SRBC) to test the antibody production or skin-sensitized with hapten picryl chloride (PCL) to induce contact hypersensitivity (CHS). In addition, the effects of opioids on the production of reactive oxygen intermediates (ROIs) and cytokines by peritoneal macrophages (Mf) and on the expression of surface markers on these cells and blood leukocytes were estimated.
Results: Opioids caused an enhancement of ROIs and cytokines production when macrophages were stimulated with zymosan or lipopolysaccharide (LPS) and reduced the expression of antigen presentation markers on Mf. Numbers of anti-SRBC plaque forming cells (PFC) and antibodies titres were lower in mice treated with all tested opioids. Depending on the use of particular opioid and the phase of allergic reaction, effects of the treatment on CHS were diverse. While morphine decreased the early and late phases of induction of CHS responses, methadone increased both reactions. In case of the effector phase of CHS, morphine and fentanyl increased both its early and late stages, while methadone decreased the late reaction. Treatment of recipients with opioids had diverse influence on the passive transfer of CHS in these animals.
Conclusions: Our experiments show that the action of opioids on the immune system is a complex phenomenon dependent on such variables as type of opioid, character of response (humoral *versus* cellular) and types of cells involved. Here Mf seem to play a significant role.

Key words:
opioids, macrophages, humoral response, contact hypersensitivity

Abbreviations: APCs – antigen presenting cells, CD – cluster of differentiation, CHS – contact hypersensitivity, DNFB – 1-fluoro-2,4-dinitrobenzene, DPBS – Dulbecco's phosphate buffered saline, FCS – fetal calf serum, FcγR – Fcγ receptor, FITC – fluorescein isothiocyanate, IL – interleukin, LPS – lipopolysaccharide, Mf – macrophages, mAb – monoclonal antibody, NK – natural killer cells, NMDA – *N*-methyl-D-aspartate, Oil-Mf – oil-induced peritoneal macrophages, OPs – opioids, OX – oxazolone, PAMPs – pathogen associated molecular patterns, PCL – picryl chloride (1,3,5-trinitrophenyl

chloride), PE – phycoerythrin, PFC – plaque forming cells, R-Mf – resident peritoneal macrophages, ROIs – reactive oxygen intermediates, SRBC – sheep red blood cells, TGF – transforming growth factor, T-Mf – thioglycollate-induced peritoneal macrophages, TNBSA – trinitrobenzene sulfonic acid, TNF – tumor necrosis factor, TNP – 2,4,6-trinitrophenyl, Treg – T regulatory cells, *vs.* – *versus*

Introduction

It is commonly known that immune cells (macrophages, neutrophils and T lymphocytes) express different types of opioid receptors [5, 6, 33] and their signalization pathways may regulate immune response. Opioid receptors are generally classified into three distinct types: μ, δ and κ [11, 37, 38]. Each class of opioid receptors is activated by appropriate endogenous opioids such as endorphins, enkephalins and dynorphins. Additionally, they have some exogenous agonists; μ receptors bind morphine, fentanyl and methadone, which is also δ agonist, and κ receptors are activated by oxycodone [5, 6, 27].

Macrophages (Mf) are engaged in nonspecific innate immunity (phagocytosis and neutralization of antigens) and in specific immune responses. They participate in the induction stage as antigen presenting cells (APCs) and as effector cells in delayed-type hypersensitivity reactions including contact hypersensitivity (CHS) [1–4]. Although Mf may act as APCs, typical antigen presentation, especially in CHS, is a complex phenomenon generally mediated by other cells, like skin dendritic cells, epidermal Langerhans cells or keratinocytes [12]. Defence functions mediated by Mf are modulated by opioid therapy [13], in most cases leading to their inhibition or reduction (phagocytosis, antibody formation). Similarly, in the presence of opioids (OPs), various immune mechanisms like activation of NK and lymphocyte proliferation under the influence of mitogens are reduced, but sometimes, although rarely, OPs (like morphine) stimulate CHS and the synthesis of interferon-γ [18–20]. These effects are enabled by the presence of cell surface opioid μ-receptors on immunocytes [6, 32, 38], which are selectively antagonized by naloxone [37]. Since Mf possess μ-opioid receptors, they potentially may be influenced by OPs [8, 24, 33, 34, 36]. Although all tested by us OPs (morphine, fentanyl and methadone) are able to activate opioid μ receptors, such action on Mf was previously shown only for morphine [20, 33, 34, 36]. All mentioned OPs vary with respect to solubility; morphine is hydrophilic while fentanyl and methadone are both lipophilic, and also differ in their pharmacokinetics [32, 37]. Fentanyl is a semisynthetic opioid which has analgesic activity 75–100 times stronger than morphine [38]. Methadone, apart from the ability to activate μ receptors, stimulates opioid δ receptors and is a NMDA-receptor agonist as well [37]. Its analgesic action depends also on the presynaptic blocking of the re-uptake of monoamines (serotonin and noradrenaline). The clinical observations confirming these particular properties of methadone lead to establishing this opioid as a basic medicament in the management of neuropathic pain [37]. Because of its lipophilic property, methadone has a long half-life time in plasma and accumulates in the tissues [38] leading to different pharmacokinetic properties and perhaps various immunomodulatory influences on immunocytes, mainly macrophages.

The aim of the present study was to examine the impact of the therapy with methadone, compared to morphine and fentanyl, on the immune functions of macrophages and other immunocytes expressing opioid receptors, in humoral and cellular immunity (contact hypersensitivity model).

Materials and Methods

Animals

Eight- to 10 week-old inbred male CBA/J mice (23 ± 2 g) from the Department of Immunology, College of Medicine, Jagiellonian University, Kraków, Poland were used. Mice had free access to standard food and water. All experiments were conducted according to the guidelines of Animal Use and Care Committee of Jagiellonian University College of Medicine (102/2009).

Reagents

The following reagents were used: lucigenin (bis-N-methylacridinum nitrate), zymosan A, hydrogen peroxide, heparin sodium salt, o-phenylenediamine, 2-mercaptoethanol (2-ME), recombinant murine TNF-α (all from Sigma, St. Louis, MO, USA); RPMI 1640