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Pathophysiology 13 (2006) 111–117

ISP PATHOPHYSIOLOGY

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Alteration in specific opioid-receptor labeling on peripheral blood leukocytes of bile duct-ligated rat

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Received 23 January 2006; accepted 6 February 2006

Abstract

Cholestasis is associated with increased tonus and activity of opioidergic system. Opioid peptides have also immunomodulatory effects through stimulation of specific opioid receptors on the immune cells, or in an indirect fashion via the central nervous system. The combination of immunofluorescent technique and flow cytometry has proven to be sensitive method for the detection of leukocyte opioid receptors. This study was designed to examine the effect of cholestasis on the opioid-receptor labeling on the leukocytes from bile duct-ligated rats. Seven days after surgery, leukocytes were isolated from the peripheral blood of bile duct-ligated or sham-operated rats. The cells were incubated with naltrexone-fluorescein, in the absence or presence of unlabeled naltrexone, as a competitor and analysed by flow cytometry. Monocytes and granulocytes from bile duct-ligated rats showed an increase in the percentage of opioid-receptor labeling (29.6 ± 2.08 for cholestatic versus 23 ± 1.9 for sham, $p < 0.001$; 50.6 ± 3.18 for cholestatic versus 39.6 ± 1.7 for sham, $p < 0.05$; respectively). Furthermore, there was a decrease in the expression of opioid receptors on leukocytes due to cholestasis. In conclusion, changes in specific opioid-receptor labeling and percent of labeled leukocytes indicate that endogenous opioid-receptor interaction may be altered in peripheral blood leukocytes in acute cholestasis.

Keywords: Peripheral blood leukocytes; Opioid receptor; Direct immunofluorescence; Rat; Cholestasis

1. Introduction

Endogenous opioid peptides are known to circulate in low levels in the plasma of mammals including humans and rats [1,2]. Many researchers have described a marked elevation of endogenous opioid levels in plasma of patients with cholestatic liver diseases and animal models of cholestasis [2–6]. Based on accumulated evidence, it has been suggested that endogenous opioids are implicated in the pathophysiology of cholestasis [2,7,8]. Observations compatible with this hypothesis include precipitation of an opioid withdrawal-like syndrome in patients with chronic cholestatic liver disease by administration of an opioid antagonist [3,4] and a global down-regulation of central mu-opioid receptors in the cholestatic rats [9].

Sibinga and Goldstein have demonstrated the presence of opioid receptors on cells of the immune system [10]. Opiates and opioid peptides induce an enhancement or a suppression of immune function, such as macrophage phagocytosis [11] and monocyte chemotaxis [12] depending on concentration

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doi:10.1016/j.pathophys.2006.02.002

and/or class of opioid used, as well as the type and/or activation/differentiated status of effector cells monitored. In addition, many researchers demonstrated that cholestasis is associated with abnormality of immune functions. The examples are disturbed neutrophil adhesion [13], chemotaxis and phagocytosis [14], in cholestatic rats, and also abnormal T-lymphocyte responses in primary biliary cirrhosis [15].

Although the characterization of brain opioid receptors has generally involved radioligand and competition binding assays with various selective opioid compounds, it has been difficult to identify classical opioid receptors on leukocytes by using radioligand binding assay [16]. This is probably because of very low opioid-receptor expression on immune cells. In addition only a small proportion of cells within heterogenous leukocyte population express opioid receptors [17]. Over two decades ago, a new method for studying opioid-receptor expression was described by Kolb et al. [18,19]. They synthesized three fluorescent-conjugated opioid ligands including naltrexone, naloxone, and oxymorphone, and demonstrated their high affinity at rat brain synaptosomal plasma membrane and biological activity at the guinea pig ileum. Then, many researchers have studied opioid receptors on isolated cells, using these probes [20,21]. As an increased plasma endogenous opioid activity has been demonstrated in cholestasis, it has been proposed that relative opioid-receptor density on the circulating leukocytes might also change. A previous study reported a change in the proportion of leukocytes with opioid receptors in the cholestatic rats. Specific opioid-receptor labeling (i.e. the relative density of opioid receptors) on leukocytes of cholestatic animals, however, was not evaluated [22].

The present study was carried out to evaluate whether specific opioid-receptor labeling on peripheral immune cells was changed in a rat model of acute cholestasis.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats weighing 220–240 g (purchased from the Pasteur Institute of Iran) were used in this study. The animals were housed in temperature controlled room ($24 \pm 1\,^{\circ}C$) on a 12-h light: 12-h dark cycle for at least 1 week before experimentation. Animals had a free access to food and water. All procedures were carried out in accordance with recommendations of the Ethics Committee on Animal Experiments of the Medical School (Tehran University of Medical Sciences, Tehran, Iran). A midline laparatomy was performed under general anaesthesia, induced by an intraperitoneal injection of ketamine (50 mg/kg) and promazine (10 mg/kg). In the bile duct-ligated (BDL) group the bile duct was isolated and doubly ligated by using method of Cameron and Oakley [23]. In sham-operated group, the bile duct was identified and manipulated without ligation. Seven days after recovery from operation, when the BDL group had shown signs of overt cholestasis (jaundice, dark urine and steatorrhea), midline laparatomy and blood sampling from the inferior vena cava were performed under general anaesthesia (as described previously) and the serum bilirubins level were determined by using commercially available kits (Zist-Shimi, Tehran, Iran).

2.2. Chemicals

Chemicals used were obtained as follows: dextran sulfate sodium (MW: 500,000 kDa) ICN Biochemical (Costa Mesa, CA, USA); naltrexone hydrochloride, Sigma Chemical Co. (St. Louis, MO, USA); naltrexone-fluorescein isothiocyanate (naltrexone-FITC) (Molecular Probes, The Netherlands). Two last drugs were dissolved in Krebs' buffer containing NaCl, 115 mmol/L; KH_2PO_4, 2 mmol/L; $MgCl_2$, 2.4 mmol/L; $NaHCO_3$, 25 mmol/L; KCl, 8 mmol/L; and $CaCl_2$, 1.3 mmol/L.

2.3. Leukocyte isolation

Leukocyte isolation was performed in accordance with the method of Boyum [24]. Briefly, 6 mL of fresh heparinized blood were diluted with two volumes of isotonic saline solution, and divided in two tubes each containing 1.5 mL dextran 6% (w/v) in NaCl 0.9% (w/v). The mixture was incubated at room temperature for 45 min to allow aggregation and sedimentation of the erythrocytes. The leukocyte rich supernatant was collected and washed (centrifuged at $200 \times g$ for 15 min at $4\,^{\circ}C$) with phosphate-buffered saline (PBS, pH 7.4). The washing was repeated once more. The contaminating erythrocytes were lysed by ice-cold isotonic ammonium chloride (NH_4Cl) solution, as necessary. Then, cells were washed three times as above at $200 \times g$ for 5 min at $4\,^{\circ}C$ with Krebs' buffer (pH 7.4). The differential cell counts were determined by a hemocytometer. The viability of the cells was tested by trypan blue exclusion.

2.4. Direct fluorescence labeling of opioid receptors

By using the naltrexone-FITC as an opioid probe, labeling of opioid receptors was performed as described previously [20]. Briefly, in a final assay volume of 60 μL of Krebs' buffer, 5×10^5 cells were incubated with 20 μL of 2–8 μM naltrexone-FITC for 30 min at $25\,^{\circ}C$ and in the dark, for determination of optimal staining of opioid receptors. The opioid-receptor antagonist naltrexone in 20 μL volume also was titrated (0.4–8 mM) to obtain the optimal concentration necessary for measurement of nonspecific fluorescence. The control sample consisted of leukocytes incubated with 40 μL Krebs, buffer alone. The assay was performed in triplicate. Samples were chilled on ice, diluted with 0.5 mL Krebs' buffer and centrifuged at $200 \times g$ for 3 min at $4\,^{\circ}C$. The supernatant was aspirated and cells were washed twice more and resuspended in a final volume of 0.5 mL of Krebs' buffer for the flow cytometric analysis.