Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 467 (2003) 81–84
www.elsevier.com/locate/ejphar

Short communication

Activation of the κ-opioid receptor in Caco-2 cells decreases interleukin-8 secretion

Brien L. Neudeck*, Jennifer Loeb, Jessica Buck

University of Wisconsin School of Pharmacy, 777 Highland Avenue, Madison, WI 53705-2222, USA

Received 16 January 2003; received in revised form 14 March 2003; accepted 18 March 2003

Abstract

The immunomodulatory effects of κ-opioid agonists at the intestinal epithelial level are not well characterized. In the present study, we determined that Caco-2 cells express the κ-opioid receptor and its activation by *trans*-(±)-3,4-dichloro-*N*-methyl-*N*[2-(1-pyrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate (U-50488) leads to decreased interleukin-8 secretion in the presence of interleukin-1β. These effects were detected over a wide range (10 nM–50 μM) of U-50488 concentrations and were reversible using the κ-opioid receptor antagonist nor-binaltorphimine. Our data suggest that activation of κ-opioid receptors on Caco-2 cells decreases interleukin-8 secretion and thus may alter the chemotactic stimulus at the epithelial level.

Keywords: Caco-2 cell; Interleukin-8; κ-Opioid receptor agonist; Opioid receptor

1. Introduction

Opioids that act via the classical κ-opioid receptor have been investigated as an alternative to μ-opioid receptor binding compounds. Reported advantages include antinociceptive effects in animal models without the undesirable side effects such as respiratory depression and dependence (Butelman et al., 2001). However, similar to μ-opioid receptor agonists, κ-opioid receptor agonists can negatively affect immune function. Previous studies have shown that κ-opioid receptor agonists decrease interleukin-1β, interleukin-17, interleukin-6, tumor necrosis factor-α, and transforming growth factor-β (Alicea et al., 1996; Belkowski et al., 1995; Bush et al., 2001). Most investigations regarding the immune-mediated effects of κ-opioids utilize circulating immune cells such as B and T lymphocytes, and macrophages (Alicea et al., 1996; Morgan, 1996; Zhang and Rogers, 2000). There is a paucity of information regarding the effects of κ-opioid receptor agonists on other cells important to the immune response such as the intestinal epithelium.

The intestinal epithelium plays a central role in mucosal immunity and is the first line of defense against pathogenic bacteria and food antigens. One mechanism by which intestinal epithelial cells participate in mucosal immunity is the release of chemokines such as interleukin-8 (Jijon et al., 2002; Thorpe et al., 2001). Interleukin-8 secreted by intestinal epithelial cells serves as an important chemotactic signal leading to the influx of numerous immune cells located in the intestine. Therefore, if κ-opioids alter interleukin-8 secretion by intestinal epithelial cells, the response to luminal inflammatory insults could be adversely affected.

In this study, we tested the hypothesis that the highly selective κ-opioid receptor agonist, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*[2-(1-pyrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate (U-50488), alters interleukin-8 secretion in Caco-2 cells. We found that Caco-2 cells express κ-opioid receptors and that their activation by U-50488 decreased interleukin-8 secretion in the presence of an inflammatory stimulus. Decreases in interleukin-8 secretion by U-50488 were reversed using a κ-opioid receptor specific antagonist.

2. Materials and methods

2.1. Cell culture

The human intestinal epithelial cell line Caco-2 was obtained from American Type Culture Collection (Rock-

* Corresponding author. Tel.: +1-608-265-1168; fax: +1-608-265-5421.
E-mail address: blneudeck@pharmacy.wisc.edu (B.L. Neudeck).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01633-9

ville, MD) and incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Growth media consisted of Dulbecco's modified Eagle's media supplemented with 25 mM glucose (Mediatech, Herndon, VA), 10% fetal bovine serum, 1% nonessential amino acids, 2 mM L-glutamine, 1 mM sodium-pyruvate, and penicillin/streptomycin (100 U/100 μg/ml) (Sigma, St. Louis, MO). Near-confluent monolayers were subcultured 1:5 approximately every 7 days using 0.25% trypsin and 0.2% ethylenediaminetetraacetic acid. Cells of passage 71–77 were used in the experiments and all experiments were conducted using cells 1–2 post-confluence.

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) detection of κ-opioid receptor mRNA

Total RNA was extracted from Caco-2 cells seeded in 60-mm tissue culture dishes using the Ultraspec II RNA isolation system (Biotecx, Houston, TX) according to manufacturer's instructions. Two micrograms of RNA was reverse-transcribed to cDNA using 25 U AMV reverse transcriptase, 40 U RNAsin, and 4000 μM dNTPs (Promega, Madison, WI) per 25 μl reaction for 60 min at 42 °C. κ-opioid receptor PCR was performed by a modification of the method by Xie et al. (1999). Five-microliter aliquots of cDNA were added to a cocktail containing PCR buffer, 1.5 mM $MgCl_2$, 800 μM dNTPs, 0.625 U Taq polymerase (Promega), and 10 pmol of 5′ and 3′ primers for a total volume of 50 μl. The κ-opioid receptor primers (University of Wisconsin Biotechnology Center, Madison, WI) were: forward 5′-AGATACACAAAGATGAAGACAGCAACCAAC-3′ reverse 5′-TCCCTGACTTTGGTGCCTCCAAGGACTATT-3′ (product length 352 bp). PCR reactions were performed in a MiniCycler thermal cycler (MJ Research, Waltham, MA) over 55 cycles with a denaturing temperature of 94 °C for 30 s followed by annealing at 56 °C for 1 min and extension at 72 °C for 1.5 min. RNA and cDNA were omitted as negative controls for the RT and PCR steps, respectively. Positive controls were performed using RNA extracted from human peripheral blood lymphocytes. Agarose gel electrophoresis (2%) was used to identify specific PCR products.

2.3. Immunocytochemical localization of κ-opioid receptors

Caco-2 cells were seeded on porous (0.4 μm) polycarbonate membrane filters (Transwell™; Costar, Cambridge, MA) at a density of 100,000/cm^2 growth area. Single-labeling immunofluorescent detection of κ-opioid receptors on Caco-2 cells was accomplished using affinity purified goat polyclonal immunoglobulin G antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). These antibodies recognize the extracellular amino terminus of the human κ-opioid receptor-1. The apical membranes were washed with 2 mg/ml bovine serum albumin in phosphate-buffered saline (PBS) (Sigma) followed by blocking with bovine serum albumin/PBS solution. Subsequently, membranes were incubated for 1 h with the primary antibody diluted 1:50 in bovine serum albumin/PBS. Following the primary antibody incubation, the membranes were washed with the bovine serum albumin/PBS solution and incubated for 1 h with a fluorescein isothiocyanate (FITC)-labeled mouse, anti-goat secondary antibody (1:50 dilution) in PBS with 1.5% bovine serum albumin (Sigma). Lastly, membranes were washed with bovine serum albumin/PBS solution and fixed with 3% paraformaldehyde in PBS at room temperature. Negative controls were conducted by omitting the primary antibody. With the exception of fixation, all steps were carried out at 4 °C. After fixation, membranes were excised, placed on a glass slide, and secured with a cover slip. Immunofluorescence was observed with a laser scanning confocal fluorescent microscope (MRC-1024, Bio-Rad Laboratories, Richmond, CA).

2.4. Measurement of interleukin-8 in Caco-2 cell supernatants

For the measurement of interleukin-8 secretion, Caco-2 cells were seeded in 24 well tissue culture plates at a density of 200,000/well. Post-confluent cell monolayers were washed twice with prewarmed PBS and allowed to equilibrate in serum-free media for 20 min. To determine if U-50488 altered interleukin-8 secretion in Caco-2 cells, cells were pre-incubated with serum-free media or U-50488 in serum-free media for 60 min. Concentrations of 10 and 100 nM, and 1, 10, and 50 μM were employed. Subsequently, the U-50488-containing media was replaced with U-50488 plus interleukin-1β (3 ng/ml) (Alexis Biochemicals, San Diego, CA) for 5 h. Cytokine production was also characterized with interleukin-1β alone. To confirm that altered interleukin-8 secretion was due to κ-opioid receptor binding, the selective κ-opioid receptor antagonist nor-binaltorphimine (10 μM) (Tocris) was employed. Nor-binaltorphimine was added 30 min prior to U-50488 and the experiments previously described repeated. Interleukin-8 secretion was also measured with nor-binaltorphimine alone and in combination with interleukin-1β. Each experimental condition was conducted in quadruplicate. Quantification of interleukin-8 was performed using a commercially available enzyme-linked immunosorbent assay according to the manufacturer's instructions (OptEIA, Pharmingen, San Diego, CA).

2.5. Statistical evaluation

Results for interleukin-8 concentrations in the cell culture supernatants are expressed as mean ± S.D. Statistical analysis of mean interleukin-8 concentrations of control cells and the treatment groups was accomplished using analysis of variance (ANOVA). For pairwise comparisons of the different treatment groups, a post hoc

Tukey test was employed using $p < 0.05$ as the level of significance.

3. Results

3.1. κ-Opioid receptors are constitutively expressed on the apical surface of Caco-2 cells

Prior to resolving the question of κ-opioid agonist mediated effects on interleukin-8 secretion, it was first necessary to determine if Caco-2 cells express the κ-opioid receptor. Using RT-PCR, Caco-2 cells were found to constitutively express κ-opioid receptors (Fig. 1A). These findings were confirmed by immunocytochemistry (Fig. 1B). Using confocal microscopy, κ-opioid receptors were visualized throughout the apical surface of the cell.

3.2. Activation of κ-opioid receptors results in decreased interleukin-8 secretion

Pre-incubation of confluent Caco-2 cells with the κ-opioid receptor agonist, U-50488 significantly decreased interleukin-8 secretion in the presence of the inflammatory stimulus, interleukin-1β (Fig. 2A). Decreased interleukin-8 secretion was observed with all concentrations of U-50488 employed. To confirm that the decreased interleukin-8 production was mediated by the κ-opioid receptor, cells were pre-incubated with the κ-opioid receptor specific antagonist nor-binaltorphimine. The addition of nor-binaltorphimine successfully blocked the decreased interleukin-8 secretion for all U-50488 concentrations with the exception of the higher, 10 and 50 μM concentrations (Fig. 2B). Neither U-50488 or nor-binaltorphimine alone altered basal interleukin-8 production (data not shown).

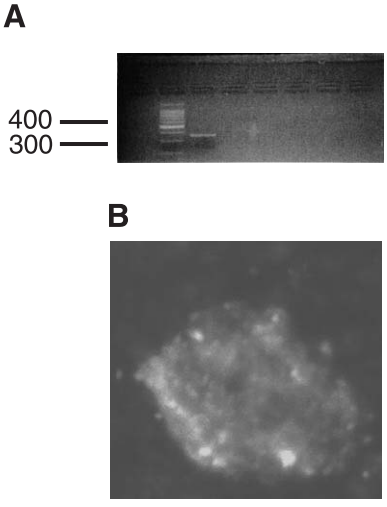

Fig. 1. Identification of κ-opioid receptor on Caco-2 cells. Panel A: expression of κ-opioid receptor mRNA in Caco-2 cells determined by RT-PCR. Panel B: localization of κ-opioid receptors on the apical surface of Caco-2 cells. Cells were incubated with a FITC-conjugated monoclonal antibody to the κ-opioid receptor and identified using confocal microscopy.

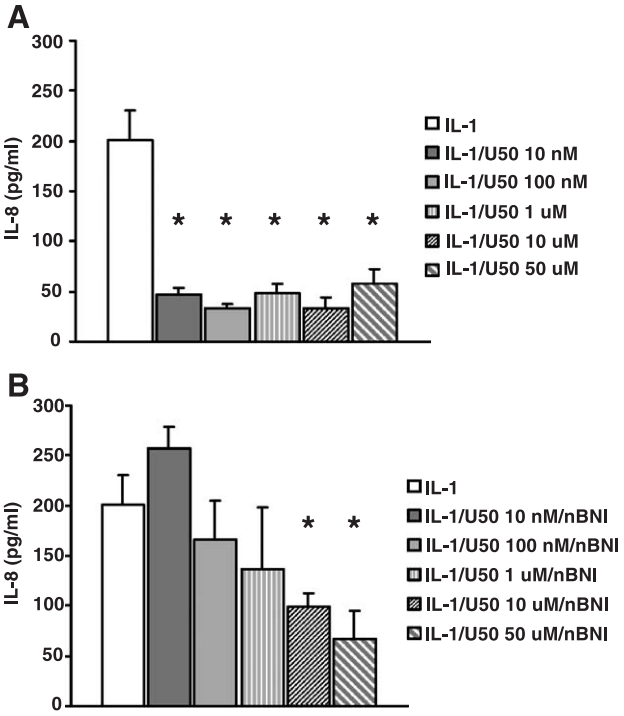

Fig. 2. Effects of U-50488 on interleukin-1β stimulated interleukin-8 secretion. Data presented as mean ± S.D. of quadruplicate experiments. (A) Cells were pre-incubated with U-50488 (10 and 100 nM, and 1, 10, and 50 μM) prior to stimulation with interleukin-1β. (B) Nor-binaltorphimine (10 μM) was added 30 min prior to U-50488 to confirm that decreased interleukin-8 secretion was mediated by the κ-opioid receptor. *$p < 0.01$ versus interleukin-1β alone.

4. Discussion

In this study, activation of the κ-opioid receptor by U-50488 decreased interleukin-8 secretion in Caco-2 cells. Involvement of the κ-opioid receptor was confirmed using nor-binaltorphimine to block U-50488-mediated decreases in interleukin-8 secretion. To our knowledge, this is the first study to demonstrate that κ-opioid receptors are present in a human intestinal epithelial cell line and their activation alters chemokine secretion.

Significant decreases in interleukin-8 secretion were noted already at 10 nM of U-50488 and across a wide range (10 nM−50 μM) of concentrations. The κ-opioid specific antagonist nor-binaltorphimine was able to block these effects for all concentrations employed except for the 10 and 50 μM U-50488 concentrations. Given that the concentration of the antagonist was only 10 μM, this is not entirely surprising and confirms a dose−response relationship for the antagonist. Frequently, nanomolar concentrations are employed when studying opioid-mediated effects on immune function (Alicea et al., 1996; Belkowski et al., 1995). Although the use of traditionally low nanomolar

concentrations yields important information regarding the sensitivity of the effects, employing higher micromolar concentrations is also justified. Micromolar opioid concentrations better reflect those achieved in the small intestine following oral administration. Moreover, employing a wide range of concentrations allows for the comparison of the sensitivity of the specific receptor on epithelial cells to other epithelial expressed opioid receptors and to receptors located in other tissues. To this end, we have previously shown that Caco-2 cells also express μ-opioid receptors and that only micromolar concentrations of the agonist led to increased interleukin-8 secretion (Neudeck and Loeb, 2002).

In summary, our results indicate that Caco-2 cells constitutively express functional κ-opioid receptors and their activation in the presence of an inflammatory stimulus results in a diminished interleukin-8 response. Decreased interleukin-8 secretion was antagonized using nor-binaltorphimine, the κ-opioid receptor specific antagonist. These findings lend credence to the hypothesis that the immunosuppressive effects of opioids may not be limited to circulating immune cells. Greater understanding of the effects of opioids on the intestinal epithelial cell immune response is required.

References

Alicea, C., Belkowski, S., Eisenstein, T.K., Adler, M.W., Rogers, T.J., 1996. Inhibition of primary murine macrophage cytokine production in vitro following treatment with the kappa-opioid agonist U50, 488H. J. Neuroimmunol. 64, 83–90.

Belkowski, S.M., Alicea, C., Eisenstein, T.K., Adler, M.W., Rogers, T.J., 1995. Inhibition of interleukin-1 and tumor necrosis factor-alpha synthesis following treatment of macrophages with the kappa opioid agonist U50, 488H. J. Pharmacol. Exp. Ther. 273, 1491–1496.

Bush, K.A., Kirkham, B.W., Walker, J.S., 2001. The kappa-opioid agonist, asimadoline, alters cytokine gene expression in adjuvant arthritis. Rheumatology 40, 1013–1021.

Butelman, E.R., Ko, M.C., Traynor, J.R., Vivian, J.A., Kreek, M.J., Woods, J.H., 2001. GR89,696: a potent kappa-opioid agonist with subtype selectivity in rhesus monkeys. J. Pharmacol. Exp. Ther. 298, 1049–1059.

Jijon, H.B., Panenka, W.J., Madsen, K.L., Parsons, H.G., 2002. MAP kinases contribute to IL-8 secretion by intestinal epithelial cells via a posttranscriptional mechanism. Am. J. Physiol., Cell Physiol. 283, C31–C41.

Morgan, E.L., 1996. Regulation of human B lymphocyte activation by opioid peptide hormones. Inhibition of IgG production by opioid receptor class (mu-, kappa-, and delta-) selective agonists. J. Neuroimmunol. 65, 21–30.

Neudeck, B.L., Loeb, J.M., 2002. Endomorphin-1 alters interleukin-8 secretion in Caco-2 cells via a receptor mediated process. Immunol. Lett. 84, 217–221.

Thorpe, C.M., Smith, W.E., Hurley, B.P., Acheson, D.W., 2001. Shiga toxins induce, superinduce, and stabilize a variety of C-X-C chemokine mRNAs in intestinal epithelial cells, resulting in increased chemokine expression. Infect. Immun. 69, 6140–6147.

Xie, G.X., Meuser, T., Pietruck, C., Sharma, M., Palmer, P.P., 1999. Presence of opioid receptor-like (ORL1) receptor mRNA splice variants in peripheral sensory and sympathetic neuronal ganglia. Life Sci. 64, 2029–2037.

Zhang, L., Rogers, T.J., 2000. Kappa-opioid regulation of thymocyte IL-7 receptor and C-C chemokine receptor 2 expression. J. Immunol. 164, 5088–5093.