Available online at www.sciencedirect.com
ScienceDirect
Drug and Alcohol Dependence 88 (2007) 36–41
DRUG and ALCOHOL DEPENDENCE
www.elsevier.com/locate/drugalcdep

Rapid heterologous desensitization of antinociceptive activity between mu or delta opioid receptors and chemokine receptors in rats

Xiaohong Chen [a,*], Ellen B. Geller [a], Thomas J. Rogers [a,b,c], Martin W. Adler [a]

[a] *Center for Substance Abuse Research, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, PA 19140, United States*
[b] *Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140, United States*
[c] *Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, United States*

Received 10 January 2006; received in revised form 6 September 2006; accepted 15 September 2006

Abstract

Previous studies have shown pretreatment with chemokines CCL5/RANTES (100 ng) or CXCL12/SDF-1alpha (100 ng) injected into the periaqueductal grey (PAG) region of the brain, 30 min before the mu opioid agonist DAMGO (400 ng), blocked the antinociception induced by DAMGO in the in vivo cold water tail-flick (CWT) antinociceptive test in rats. In the present experiments, we tested whether the action of other agonists at mu and delta opioid receptors is blocked when CCL5/RANTES or CXCL12/SDF-1alpha is administered into the PAG 30 min before, or co-administered with, opioid agonists in the CWT assay. The results showed that: (1) CXCL12/SDF-1alpha (100 ng, PAG) or CCL5/RANTES (100 ng, PAG), given 30 min before the opioid agonist morphine, or selective delta opioid receptor agonist DPDPE, blocked the antinociceptive effect of these drugs; (2) CXCL12/SDF-1alpha (100 ng, PAG) or CCL5/RANTES (100 ng, PAG), injected at the same time as DAMGO or DPDPE, significantly reduced the antinociceptive effect induced by these drugs. These results demonstrate that the heterologous desensitization is rapid between the mu or delta opioid receptors and either CCL5/RANTES receptor CCR5 or CXCL12/SDF-1alpha receptor CXCR4 in vivo, but the effect is greater if the chemokine is administered before the opioid.

Keywords: Chemokine receptors; Chemokine ligands; Opioid receptors; Opioid agonists; Heterologous desensitization; Rat

1. Introduction

The chemokines are a family of chemoattractant cytokines. Chemokines and their receptors are present throughout the brain, and their function is under intense study. They have been reported to be involved in communication between neurons and glia (Dorf et al., 2000), central nervous system development (Zou et al., 1998), neuronal survival (Zhang et al., 1998), tumor angiogenesis and/or inhibition of immune response (Rempel et al., 2000), the response to inflammatory insults, such as lipopolysaccharide (Banisadr et al., 2002), human immunodeficiency virus (HIV) infection through coreceptors (Gerard and Rollins, 2001; Moore, 1997; Proudfoot et al., 1999), as well as therapies for HIV infection (Howard et al., 1999; Moore, 1997; Proudfoot et al., 1999). In addition to those chemokine activities, recent evidence demonstrates that the analgesic function of mu opioids in the brain (Szabo et al., 2002) and pain sensitivity can also be modulated by the release of chemokines in the spinal cord (Boddeke, 2001). The role of chemokines in the function of the central nervous system has recently been reviewed (Adler et al., 2006; Adler and Rogers, 2005). Both the opioids and chemokines mediate their effects on leukocytes through the activation of G-protein-coupled seven transmembrane receptors. Three major types of opioid receptors have been cloned, designated mu, delta and kappa (Chen et al., 1993; Evans et al., 1992; Kieffer et al., 1992; Li et al., 1993; Yasuda et al., 1993). There are four families of chemokine receptors, designated C, CC, CXC and CX3C (Murphy et al., 2000). In this terminology, "L" refers to a ligand and "R" to a receptor. Hence, CCL5/RANTES (regulated on activation normal T cell expressed and secreted) is a ligand in the CC family and CXCL12/SDF-1alpha (stromal cell-derived factor-1alpha) belongs to the CXC family. Some chemokines, like CCL5/RANTES, can bind to more than one chemokine receptor, but others, like CXCL12/SDF-1alpha and CXC3L1/fractalkine, are specific to one receptor.

Heterologous desensitization occurs when a G-protein-coupled receptor (GPCR), activated by an agonist, initiates a signaling process leading to the inactivation (desensitization)

* Corresponding author. Tel.: +1 215 707 5305; fax: +1 215 707 1904.
E-mail address: chenyang@temple.edu (X. Chen).

0376-8716/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.drugalcdep.2006.09.010

of an unrelated GPCR. A study by Szabo et al. (2002) has shown that CCL5/RANTES treatment of leukocytes, leukocyte cell lines or cell lines transfected with CCR5 and the mu opioid receptor abolished the chemotactic response to [d-Ala2, N-Me-Phe4, Gly5-ol]enkephalin (DAMGO) and vice versa. Heterologous desensitization between opioid receptors (mu and delta) and the chemokine CCL5/RANTES receptor(s) or the chemokine CXCL12/SDF-1alpha receptor, CXCR4, has been reported in vivo and in vitro in chemotaxis and the regulation of opioid antinociception in rats (Grimm et al., 1998; Homan et al., 2002; Rogers and Peterson, 2003; Rogers et al., 2000; Shen et al., 2000; Steele et al., 2002; Szabo and Rogers, 2001; Szabo et al., 2001a,b, 2002, 2003; Zhang et al., 2003, 2004).

Morphine is a widely used analgesic that has its primary action on mu opioid receptors. DAMGO and [D-Pen2, D-Pen5]-enkephalin (DPDPE) are synthetic opioid peptides that are selective for mu and delta opioid receptors, respectively (Handa et al., 1981; Mosberg et al., 1983). On the basis of previous studies showing the capacity of chemokines to desensitize mu opioid receptor function, the aim of the present experiments was to investigate whether the chemokines CCL5/RANTES or CXCL12/SDF-1alpha microinjected into the periaqueductal grey (PAG) region of the brain, a primary center for pain perception, could affect mu or delta opioid antinociceptive activity in the cold water tail-flick (CWT) test and whether desensitization occurred immediately.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Zivic-Miller), weighing 175–200 g, were housed in groups of three to four for at least 1 week in an animal room maintained at 22 ± 1 °C and approximately 50 ± 5% relative humidity. Lighting was on a 12-h light:12-h dark cycle (lights on at 7:00 h and off at 19:00 h). Rats were allowed free access to food and water.

2.2. Surgery procedures

Animals were anesthetized with a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). A sterilized stainless steel C313G cannula guide (22 gauge, Plastic One) was implanted into the PAG and fixed with dental cement. The stereotaxic coordinates are as follows: 7.8 mm posterior to bregma, 0.5 mm from midline and 5 mm ventral to the dura mater (Paxinos and Watson, 1998). A C313DC cannula dummy (Plastic One) of the identical length was inserted into the guide tube to prevent its occlusion. The animals were housed individually after surgery. Experiments began 1 week postoperatively. Each rat was used only once. At the end of the experiment, cannula placements were verified using microinjection of 1% bromobenzene blue according to the standard procedures in our laboratory (Handler et al., 1994).

2.3. Nociceptive test

The latency to flick the tail in cold water was used as the antinociceptive index, according to a standard procedure in our laboratory (Pizziketti et al., 1985). A 1:1 mix of ethylene glycol:water was maintained at −3 °C with a circulating water bath (Model 9500, Fisher Scientific; Pittsburgh, PA). Rats were held over the bath with their tails submerged approximately half-way into the solution. All animals were tested at 60, 15 and 0 min before drug injection. For each animal, the first reading was discarded and the mean of the second and third readings was taken as the baseline value. Latencies to tail flick after injection were expressed as percentage change from baseline. The percent of maximal possible antinociception (MPA%) for each animal at each time was calculated using the formula: MPA% = [(test latency − baseline latency)/(60 − baseline latency)] × 100. A cutoff limit of 60 s was set to avoid damage to the tail.

2.4. Drugs

Morphine was obtained from the Research Triangle Institute. DAMGO and DPDPE were made by Multiple Peptide Systems, San Diego, CA. These drugs were dissolved in the 0.9% saline.

The chemokines CCL5/RANTES and CXCL12/SDF-1alpha were obtained from R&D Systems, Inc. The CCL5/RANTES we used is a DNA sequence encoding the mature mouse CCL5/RANTES protein sequence (amino acid residues 24–91 of the precursor protein) (Schall et al., 1992). The CXCL12/SDF-1alpha is a DNA sequence encoding the mature mouse CXCL12/SDF-1alpha protein sequence (amino acid residues 22–89) (Nagasawa et al., 1994; Tashiro et al., 1993). These drugs were dissolved in artificial cerebrospinal fluid (aCSF) from CMA Microdialysis, MA.

2.5. Injections

One week after surgery, with aseptic procedures, a C313I internal cannula (28 gauge, Plastics One) was connected to a 10 μl Hamilton syringe by polyethylene tubing. All injections of chemokines or drugs were made into the PAG through this cannula in a volume of 1.0 μl over a 30-s period.

2.6. Statistical analysis

The data are expressed as the mean and standard error. Statistical analysis of difference between groups was assessed with a two-way analysis of variance (ANOVA) followed by Duncan's test. $P \leq 0.05$ was taken as the significant level of difference.

3. Results

3.1. Antinociception induced by injection of morphine or DPDPE into the PAG is blocked by pretreatment with CCL5/RANTES injected into the same site

Rats were divided into three groups as follows: aCSF + morphine, CCL5/RANTES + saline and CCL5/RANTES + morphine. They were given an injection of CCL5/RANTES (100 ng) or aCSF into PAG 30 min before PAG injection of morphine (100 ng, Fig. 1A). Rats that received morphine alone showed the expected antinociception. CCL5/RANTES (100 ng) blocked the antinociceptive effect induced by morphine. These results demonstrate that heterologous desensitization between the mu opioid receptors and CCL5 receptors in vivo occurred when the chemokine was administered 30 min before the mu opioid agonist (morphine).

In the next experiment, the delta opioid receptor agonist DPDPE (100 ng, PAG) was used instead of morphine as in the previous series of experiments. Similar results were observed (Fig. 1B). Rats that received DPDPE alone showed the expected antinociception. CCL5/RANTES (100 ng) blocked the antinociceptive effect induced by DPDPE. These results demonstrate that heterologous desensitization between the delta opioid receptors and CCL5 receptors in vivo occurred when the chemokine was administered 30 min before the delta opioid agonist (DPDPE).