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RAPID HETEROLOGOUS DESENSITIZATION OF ANTINOCICEPTIVE ACTIVITY BETWEEN MU OR DELTA OPIOID RECEPTORS AND CHEMOKINE RECEPTORS IN RATS

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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 minutes (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

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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 CXCL11/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) of an unrelated GPCR. A study by Szabo et al. (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 et al. 2002; Szabo and Rogers, 2001; Szabo et al. 2001a; Szabo et al. 2001b; Szabo et al. 2003; Zhang et al. 2003; Zhang et al. 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 3–4 for at least 1 week in an animal room maintained at 22±1 °C and approximately 50±5% relative humidity. Lighting was on a 12/12 h light/dark cycle (lights on at 7:00 and off at 19:00). 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: $\% \text{MPA} = [(\text{test latency} - \text{baseline latency}) / (60 - \text{baseline latency})] \times 100$. 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-1 α 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-1 α is a DNA sequence encoding the mature mouse CXCL12/SDF-1 α 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-second 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 3 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, Figure 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 (Figure 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).

3.2. Antinociception induced by injection of morphine or DPDPE into the PAG is blocked by pretreatment with CXCL12/SDF-1alpha injected into the same site

Rats were divided into 3 groups as follows: aCSF + morphine, CXCL12/SDF-1alpha + saline and CXCL12/SDF-1alpha + morphine. They were given an injection of CXCL12/SDF-1alpha (100 ng) or aCSF into PAG 30 min before injection of morphine (100 ng, Figure 2A). The results in figure 2A showed that CXCL12/SDF-1alpha significantly reduced the antinociceptive effect induced by the mu opioid receptor agonist morphine. In the next experiment, the design was similar to that in the previous one, except for substitution of the mu opioid receptor agonist morphine by the delta opioid receptor agonist DPDPE (100 ng) (Figure 2B). The result shown in figure 2B demonstrates that CXCL12/SDF-1alpha significantly reduced the antinociceptive effect induced by the delta opioid receptor agonist DPDPE.

As was the case with CCL5, heterologous desensitization between the mu or delta opioid receptors and CXCL12 receptors in vivo occurred when CXCL12/SDF-1alpha was administered 30 min before the mu or delta opioid agonists.

3.3. Antinociception induced by injection of DAMGO or DPDPE was reduced by simultaneous treatment with CCL5/RANTES injected into PAG

Rats were divided into 2 groups aCSF + DAMGO, and CCL5/RANTES + DAMGO. They were given an injection of CCL5/RANTES (100 ng) or aCSF into PAG at the same time as injection of 400 ng DAMGO. CCL5/RANTES, injected at the same time as DAMGO, significantly reduced, but did not totally block, the antinociceptive effect induced by DAMGO (shown in Figure 3A). Another experiment was conducted with rats receiving a PAG injection of CCL5/RANTES (100 ng) or aCSF at the same time as 100 ng DPDPE (Shown in Figure 3B). CCL5/RANTES (100 ng, PAG), injected at the same time as DPDPE, significantly reduced the antinociceptive effect induced by DPDPE.

These experiments show that both the mu (DAMGO)- and the delta (DPDPE)-receptor mediated antinociception is antagonized by CCL5/RANTES given at the same time, although not as effectively as when given 30 min prior to the opioid.

3.4. Antinociception induced with DAMGO or DPDPE was reduced by simultaneous treatment with CXCL12/SDF-1alpha injected into PAG

Sixteen rats were divided into 2 groups aCSF + DAMGO, and CXCL12/SDF-1alpha + DAMGO and were given an injection of CXCL12/SDF-1alpha (100 ng) or aCSF into PAG at the same time as 400 ng DAMGO (shown in figure 4A). Figure 4A shows that CXCL12/SDF-1alpha, injected at the same time as DAMGO, significantly reduced, but did not totally block, the antinociceptive effect induced by DAMGO.

Another nineteen rats were divided into 2 groups aCSF + DPDPE and CXCL12/SDF-1alpha + DPDPE and were given a PAG injection of CXCL12/SDF-1alpha (100 ng) or aCSF at the same time as 100 ng DPDPE (shown in figure 4B). The results showed that CXCL12/SDF-1alpha, injected at the same time as DPDPE, partially blocked the antinociceptive effect induced by DPDPE.

4. Discussion

Morphine is an opioid agonist that has varying degrees of activity at all opioid receptors. Pretreatment with CCL5/RANTES or CXCL12/SDF-1alpha can markedly reduce or completely block the antinociceptive effect of morphine when both agents are administered into the PAG (Figures 1 and 2), the area of the brain most involved with analgesic responses (Lewis and Gebhart, 1977; Xin et al. 1997). However, when morphine is given subcutaneously, and the chemokines are administered into the PAG, there is only a partial block of the antinociceptive effect of morphine (Adler et al. 2006). These findings demonstrate that (1) the action of morphine on the PAG is desensitized by intra-PAG administration of the chemokines; and (2) the analgesic effect of morphine outside the PAG seems not to be so effectively antagonized by intra-PAG chemokines, possibly because of peripheral effects of morphine.

DPDPE, acting selectively on the delta opioid receptor, also shows heterologous desensitization with these chemokines (Figures 1 and 2). Our laboratories were the first to report an apparent *in vivo* inactivation of opioid receptors by chemoattractant factors (Szabo et al. 2002), demonstrating that CXCL12 or CCL5, administered into the PAG, caused a dose-dependent (1 to 100 ng) reduction in DAMGO-induced antinociception over the entire 120-min duration of the experiment. The analgesic activity of the opioids in the brain presumably is antagonized in situations in which there are elevated levels of CCR5 and CXCR4 ligands, which frequently occurs during neuro-inflammatory conditions (Brack et al. 2004; Rittner and Stein, 2005; Szabo et al. 2002; Volk et al. 2004). These findings confirm that heterologous desensitization between the mu or delta opioid receptors and chemokine (CCL5/RANTES or CXCL12/SDF-1alpha) receptors exists *in vivo* (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; Szabo et al. 2001b; Szabo et al. 2003; Zhang et al. 2003; Zhang et al. 2004).

Our previous studies (Szabo et al. 2002) have shown that intra-PAG injection of CCL5/RANTES (100 ng), 30 min to 1 hr before 400 ng DAMGO administration, significantly reduced the antinociceptive effect induced by DAMGO, a selective opioid peptide agonist at mu receptors. If the pretreatment time was increased to 2 hr, there was no effect on the DAMGO antinociception. However, a second injection of CCL5/RANTES restored the heterologous desensitization. The same situation exists for CXCL12/SDF-1alpha, except that the effect is

of longer duration (2 hr or more). If the pretreatment time was increased to 4 hr, there was no effect on the DAMGO antinociception, but a second injection of CXCL12/SDF-1 α restored the heterologous desensitization. These results demonstrate that activation of the CCL5 or CXCL12 receptor causes heterologous desensitization of the μ opioid receptor in rats and is dependent on the timing of the injection, indicating that the desensitization is temporary. This may be due to degradation of the chemokines within the PAG. In the present study, the potential for rapid heterologous desensitization between the receptors for the chemokines CCL5/RANTES or CXCL12/SDF-1 α and μ or δ opioid receptors in antinociception in rats was tested. CCL5/RANTES, injected at the same time as DAMGO or DPDPE, significantly reduced, but did not totally block, the antinociceptive effect induced by DAMGO or DPDPE (shown in figure 3). Similarly, CXCL12/SDF-1 α , injected at the same time as DAMGO or DPDPE, significantly reduced, but did not totally block, the antinociceptive effect induced by DAMGO and DPDPE (shown in figure 4). Thus, the same dose of chemokine was less effective than when given 30 minutes before the opioids (shown in the figures 1B and 2B, and Szabo et al. 2002). However, these data confirm that heterologous desensitization between the μ opioid receptors and CCL5 or CXCL12 receptors in vivo can also occur when the μ or δ agonist and chemokine are administered simultaneously.

Chemokines are involved in neuronal development and various actions in the brain. The evidence of heterologous desensitization with opioid receptors suggests that the chemokine system in the brain plays an important role in the overall functioning of the brain (Adler et al. 2006; Shen et al. 2000; Szabo et al. 2001b; Zhang et al. 2003). The present results add to the evidence for heterologous desensitization between chemokine receptors and opioid receptors and reinforces our hypothesis that chemokines represent a third major system in the brain, joining neurotransmitters and neuropeptides. Potential therapeutic applications of the cross-talk among these receptors range from enhancing opioid analgesia in inflammatory pain to modifying side-effects associated with opioid therapeutics.

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Figure 1.

Antinociception induced by PAG injection of morphine (A) or DPDPE (B) blocked by pretreatment with PAG injection of CCL5/RANTES. Rats were given a PAG injection of CCL5/RANTES (100 ng) or aCSF 30 min before injection of 100 ng morphine (A) or 100 ng DPDPE (B), respectively. Each point represents the mean + SEM. N in the figure represents the number of animals used in the experiment for each group (same as below).

Figure 2.

Antinociception induced by PAG injection of morphine (A) or DPDPE (B) blocked by pretreatment with PAG injection of CXCL12/SDF-1alpha. Rats were given a PAG injection of CXCL12/SDF-1alpha (100 ng) or aCSF 30 min before injection of 100 ng morphine (A) or 100 ng DPDPE (B), respectively. Each point represents the mean + SEM.

Figure 3.

Antinociception induced by PAG injection of DAMGO (A) or DPDPE (B) reduced by simultaneous PAG injection of CCL5/RANTES. Rats were given a PAG injection of CCL5/RANTES (100 ng) or aCSF at the same time as injection of 400 ng DAMGO (A) or 100 ng DPDPE (B), respectively. Each point represents the mean + SEM.

Figure 4.

Antinociception induced by PAG injection of DAMGO (A) or DPDPE (B) reduced by simultaneous PAG injection of CXCL12/SDF-1alpha. Rats were given a PAG injection of CXCL12/SDF-1alpha (100 ng) or aCSF at the same time as injection of 400 ng DAMGO (A), or 100 ng DPDPE (B), respectively. Each point represents the mean + SEM.