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Analgesic efficacy of buprenorphine in the presence of high levels of SDF-1 α /CXCL12 in the brain

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Abstract

Although morphine is often the best option for treating acute and chronic severe pain, its analgesic activity can be blocked in situations in which there are elevated levels of chemokines. Indeed, recently we have shown that elevated brain levels of the chemokine stromal cell-derived growth factor-1 α (SDF-1 α /CXCL12, the ligand of the HIV co-receptor CXCR4) diminish the antinociceptive effect of morphine. The purpose of the present study was to investigate whether such an effect is restricted to morphine or extends to other opioid medications such as buprenorphine. A sterilized stainless-steel C313G guide cannula was implanted into the periaqueductal grey (PAG), a brain region critical to the processing of pain signals, and a primary site of action of many analgesic compounds. The cold-water (-3°C) tail-flick test (CWT) was used to measure antinociception. Rats were pretreated with SDF-1 α /CXCL12 administered into the PAG, and the antinociceptive actions of buprenorphine were measured. Direct infusion of SDF-1 α /CXCL12 into the PAG failed to alter the antinociceptive action of buprenorphine. The presence of SDF-1 α /CXCL12 in the PAG differentially alters the antinociceptive function of opioid medications. While it was able to diminish the antinociception induced by morphine (Adler et al., 2006), SDF-1 α /CXCL12 did not affect the buprenorphine-induced antinociception. Buprenorphine appears to be more effective in the presence of high levels of SDF-1 α /CXCL12 in the brain (which frequently occurs during neuroinflammatory conditions).

Keywords

Buprenorphine; Chemokine; Analgesia. PAG

1. Introduction

Buprenorphine is a semi-synthetic derivative of thebaine. It has a molecular weight of 467 and its structure is typically opioid with the inclusion of a C-7 side-chain containing a t-butyl group. Compared with morphine and methadone, which behave as full mu-opioid agonists, buprenorphine is usually defined as a mu-opioid partial agonist that shows high affinity for, and slow dissociation from, the mu-opioid receptor. Clinically, buprenorphine is an effective analgesic with a potency at least 30 times that of morphine. The lowest dose recommended for intra-muscular use (0.3 mg) has been shown to be as effective as morphine (10 mg) but has a longer duration of action (6–18 h) (Kay, 1978). Comparison of

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Conflict of interest

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the antinociceptive effects of methadone and buprenorphine shows that 3 mg/kg for methadone is the effective analgesic dose, and 0.3 mg/kg for buprenorphine in rats (Bulka et al., 2004). Respiratory depression caused by opioids can be potentially life-threatening, but is much less of a problem with buprenorphine than with many other opioids including morphine, hydromorphone, methadone, oxycodone, and transdermal fentanyl (Dahan et al., 2005). This advantage is due to buprenorphine's bell-shaped dose–response curve with regard to respiratory depression, meaning that the risk to induce respiratory arrest does not linearly follow dose-increments of the substance. This ceiling effect provides a measure of safety for the drug in clinical practice (Dahan et al., 2005). Clinically, there is also a less marked effect of buprenorphine binding to mu-opioid receptors in the gastrointestinal tract, thereby slowing transit times. Indeed, constipation seen in the clinic is remarkably low (Griessinger et al., 2005). Due to its slow dissociation from the mu receptor and the resulting milder withdrawal symptoms, the risk of the development of drug dependence and analgesic tolerance in the short- or long-term seems to be lower with buprenorphine than with full mu opioids (Heel et al., 1979; Robinson, 2002; Walsh et al., 1995; Walsh and Eissenberg, 2003).

We have developed evidence for a functional interaction between chemokine receptors, particularly those involved in the HIV life-cycle, and opioid system. Recently, we have shown that elevated levels of SDF-1 α /CXCL12 in the brain (a condition that occurs with neuroinflammatory diseases, including HIV encephalitis) reduced the antinociceptive effect of morphine (Adler et al., 2006). While as yet unexplored, the unique pharmacological characteristics of buprenorphine may make it more suitable and effective for treating pain under neuroinflammatory conditions compared to other opioid agonists, particularly morphine. Thus, the purpose of the present study was to test the antinociceptive effectiveness of buprenorphine in the presence of high levels of SDF-1 α /CXCL12 in the brain.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (ACE Animals, Inc., Boyertown, PA 19512), 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 $50 \pm 2\%$ relative humidity. Lighting was on a 12/12-h light/dark cycle (lights on at 7:00 and off at 19:00). The animals were allowed free access to food and water. All animal use procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee.

2.2. Surgery procedures

Rats were anesthetized with a mixture of ketamine hydrochloride (100–150 mg/kg) and acepromazine maleate (0.2 mg/kg). A sterilized stainless steel C313G guide cannula (22 gauge, Plastics One) was implanted into the PAG and fixed with dental cement. The stereotaxic coordinates were 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 (Plastics 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.

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 halfway 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 are expressed as percentage change from baseline. The percentage of maximal possible antinociception (%MPA) for each animal at each time was calculated using the following 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

Buprenorphine was obtained from the National Institute on Drug Abuse and were dissolved in sterile pyrogen-free saline. SDF-1 α /CXCL12 was obtained from R&D Systems (Minneapolis, MN) and was dissolved in artificial cerebrospinal fluid (CSF) from CMA Microdialysis, MA.

2.5. Injections

After a 7-day recovery period, rats were allowed to habituate to test chambers for 1 h before testing. With aseptic procedures, a C313I internal cannula (28 gauge, Plastics One) was connected to a 10 μl Hamilton syringe by polyethylene tubing. A volume of 1 μl of drug or vehicle was delivered at a rate of 1 $\mu\text{l}/\text{min}$ (manually) and the internal cannula left in place an additional 90 s to allow diffusion. Immediately thereafter, a dummy cannula (C313DC) was inserted into the cannula guide to prevent any contamination.

2.6. Statistical and histological analysis

All data are reported as means $\pm$ SEM, and the variations in %MPA were compared across treatments and time points and analyzed by two-way ANOVA followed by Bonferroni's test. The data were analyzed by Prism software (Graph-Pad, San Diego, CA). Significance was set at $P < 0.05$.

At the conclusion of the experiments, each rat was injected with 0.5 μl of cresyl violet, anesthetized and perfused transcardially with 0.9% isotonic saline, followed by phosphate-buffered saline (PBS) and 4% paraformaldehyde. The brain was removed, stored in the same fixative for 4 h, kept in 20% sucrose overnight, and cut into 20- μm sections on a freezing microtome. Each coronal section was mounted according to standard histological procedures (Benamar et al., 2004), and the site of injection was verified by locating the dye (Fig. 1B).

3. Results

After a 60-min baseline interval, SDF-1 α /CXCL12 was injected into the PAG. Thirty minutes later, buprenorphine was injected. The antinociceptive dose of buprenorphine (1 mg/kg) given subcutaneously (s.c.) produced a marked antinociception in the cold-water tail-flick test (CWT), reaching a peak level ($89 \pm 10\% \text{MPA}$) at 45 min (Fig. 1A). The onset was rapid, with an effect observed 15 min after injection. Unlike with morphine, the pretreatment with SDF-1 α /CXCL12 (100 ng, PAG) failed to alter the analgesic effect of buprenorphine (Fig. 1A, $P > 0.05$).

4. Discussion

Pain is one of the most widespread and intractable of human complaints. Its pathogenesis is immensely complex, involving structural, physiological, and pharmacological changes throughout the neuroaxis. Multiple pharmacological agents have been used to treat diverse

pathological pain states, including nonsteroidal anti-inflammatory drugs, anticonvulsants, antidepressants, opioids and others (Guindon et al., 2007). A wide variety of opioids that have different pharmacological properties are used in the clinic for the treatment of various forms of pain. The use of an opioid with analgesic properties and reduced side effects is highly desirable. Buprenorphine was approved by the FDA in 2002 for use in supervised withdrawal and maintenance treatment of opioid dependence. Buprenorphine is a mu-opioid partial agonist, a powerful analgesic in both rodents and humans, has a very long-lasting efficacy, high safety profile and does not possess immunosuppressive properties.

In the present studies, we have tested the effect of direct infusion of SDF-1 α /CXCL12 into the PAG at a dose that diminished the antinociceptive effect of morphine (Adler and Rogers, 2005) on buprenorphine-induced antinociception. Unlike the blockade of the antinociceptive effect of morphine, SDF-1 α /CXCL12 did not affect the antinociceptive action of buprenorphine. The lack of interaction between buprenorphine and the immune system has been reported previously. In a study in the PAG of the rat, the indirect neurohumoral effects of buprenorphine and morphine on the immune system were investigated. In contrast to morphine, buprenorphine did not result in any functional changes of splenic natural killer (NK) cells, T-lymphocytes or macrophages (Gomez-Flores and an Weber, 2000). Several preclinical studies clearly indicate that buprenorphine does not possess immunosuppressive properties (Franchi et al., 2007; Gomez-Flores and an Weber, 2000; Martucci et al., 2004; Sacerdote et al., 2008). The pharmacological properties of buprenorphine, i.e., its antagonism at the kappa-opioid receptor, are thought to be partly responsible for the lack of immunosuppression (Evans and Easthope, 2003).

The differential effects of buprenorphine and morphine on analgesic functions in the presence of SDF-1 α /CXCL12 in the brain may be related to their characteristic opioid-receptor binding and affinities. Overall, the body of literature suggests that buprenorphine is an opioid with unique and complex pharmacology, i.e., it can act as an agonist and/or antagonist at different classes of opioid receptors (Lutfy and Cowan, 2004). Although buprenorphine has been shown to interact *in vivo* and *in vitro* with multiple opioid receptors, its primary activity is as a partial agonist at the mu-opioid receptor and antagonist at the kappa receptor (Leander et al., 1988; Martin et al., 1976; Richards and Sadee, 1985). The majority of data shows that buprenorphine acts as a delta opioid receptor antagonist and as a partial agonist at the opioid receptor-like 1 (ORL1) receptor (Marquez et al., 2008).

Regardless of the mechanism involved, the fact that buprenorphine appears to be more effective in the presence of high levels of SDF-1 α /CXCL12 in the brain in comparison with the standard opioid agonist that does not share same of its pharmacological characteristics, namely morphine (Adler and Rogers, 2005), has great implications and is relevant for public health, particularly in pain states associated with inflammatory processes, including HIV-related pain management with opioid medications.

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References

- Adler MW, Geller EB, Chen X, Rogers TJ. Viewing chemokines as a third major system of communication in the brain. *AAPS J.* 2006; 7:E865–E870. [PubMed: 16594639]
- Adler MW, Rogers TJ. Are chemokines the third major system in the brain? *J Leukoc Biol.* 2005; 78:1204–1209. [PubMed: 16204637]

- Benamar K, Rawls SM, Geller EB, Adler MW. Intrahypothalamic injection of deltorphin-II alters body temperature in rats. *Brain Res.* 2004; 1019:22–27. [PubMed: 15306234]
- Bulka A, Kouya PF, Bottiger Y, Svensson JO, Xu XJ, Wiesenfeld-Hallin Z. Comparison of the antinociceptive effect of morphine, methadone, buprenorphine and codeine in two substrains of Sprague–Dawley rats. *Eur J Pharmacol.* 2004; 492:27–34. [PubMed: 15145702]
- Dahan A, Yassen A, Bijl H, Romberg R, Sarton E, Teppema L, Olofsen E, Danhof M. Comparison of the respiratory effects of intravenous buprenorphine and fentanyl in humans and rats. *Br J Anaesth.* 2005; 94:825–834. [PubMed: 15833777]
- Evans HC, Easthope SE. Transdermal buprenorphine. *Drugs.* 2003; 63:1999–2010. discussion 2011–2. [PubMed: 12962515]
- Franchi S, Panerai AE, Sacerdote P. Buprenorphine ameliorates the effect of surgery on hypothalamus–pituitary–adrenal axis, natural killer cell activity and metastatic colonization in rats in comparison with morphine or fentanyl treatment. *Brain Behav Immun.* 2007; 21:767–774. [PubMed: 17291715]
- Gomez-Flores R, an Weber RJ. Differential effects of buprenorphine and morphine on immune and neuroendocrine functions following acute administration in the rat mesencephalon periaqueductal gray. *Immunopharmacology.* 2000; 48:145–156. [PubMed: 10936512]
- Griessinger N, Sittl R, Likar R. Transdermal buprenorphine in clinical practice—a post-marketing surveillance study in 13,179 patients. *Curr Med Res Opin.* 2005; 21:1147–1156. [PubMed: 16083522]
- Guindon J, Walczak JS, Beaulieu P. Recent advances in the pharmacological management of pain. *Drugs.* 2007; 67:2121–2133. [PubMed: 17927280]
- Heel RC, Brogden RN, Speight TM, Avery GS. Buprenorphine: a review of its pharmacological properties and therapeutic efficacy. *Drugs.* 1979; 17:81–110. [PubMed: 378645]
- Kay B. A double-blind comparison of morphine and buprenorphine in the prevention of pain after operation. *Br J Anaesth.* 1978; 50:605–609. [PubMed: 352372]
- Leander JD, Gesellchen PD, Mendelsohn LG. Opioid antinociceptive effects of delta-receptor antagonists. *Pharmacol Biochem Behav.* 1988; 29:351–355. [PubMed: 2834753]
- Lutfy K, Cowan A. Buprenorphine: a unique drug with complex pharmacology. *Curr Neuropharmacol.* 2004; 2:395–402. [PubMed: 18997874]
- Marquez P, Borse J, Nguyen AT, Hamid A, Lutfy K. The role of the opioid receptor-like (ORL1) receptor in motor stimulatory and rewarding actions of buprenorphine and morphine. *Neuroscience.* 2008; 155:597–602. [PubMed: 18634857]
- Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther.* 1976; 197:517–532. [PubMed: 945347]
- Martucci C, Panerai AE, Sacerdote P. Chronic fentanyl or buprenorphine infusion in the mouse: similar analgesic profile but different effects on immune responses. *Pain.* 2004; 110:385–392. [PubMed: 15275790]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates.* Academic Press; San Diego: 1998.
- Pizziketti RJ, Pressman NS, Geller EB, Cowan A, Adler MW. Rat cold water tail-flick: a novel analgesic test that distinguishes opioid agonists from mixed agonist–antagonists. *Eur J Pharmacol.* 1985; 119:23–29. [PubMed: 2867920]
- Richards ML, Sadee W. In vivo opiate receptor binding of oripavines to mu, delta and kappa sites in rat brain as determined by an ex vivo labeling method. *Eur J Pharmacol.* 1985; 114:343–353. [PubMed: 2998812]
- Robinson SE. Buprenorphine: an analgesic with an expanding role in the treatment of opioid addiction. *CNS Drug Rev.* 2002; 8:377–390. [PubMed: 12481193]
- Sacerdote P, Franchi S, Gerra G, Leccese V, Panerai AE, Somaini L. Buprenorphine and methadone maintenance treatment of heroin addicts preserves immune function. *Brain Behav Immun.* 2008; 22:606–613. [PubMed: 18294814]
- Walsh SL, Eissenberg T. The clinical pharmacology of buprenorphine: extrapolating from the laboratory to the clinic. *Drug Alcohol Depend.* 2003; 70:S13–27. [PubMed: 12738347]

Walsh SL, June HL, Schuh KJ, Preston KL, Bigelow GE, Stitzer ML. Effects of buprenorphine and methadone in methadone-maintained subjects. *Psychopharmacology (Berl)*. 1995; 119:268–276. [PubMed: 7675960]

Fig. 1.
 (A) SDF-1α/CXCL12 fails to alter the antinociception induced by s.c. buprenorphine. SDF-1α/CXCL12 was given into the PAG 30 min before buprenorphine. *N*, number of rats. %MPA indicates the maximum percent analgesia. Each point represents the mean $\pm$ SEM. Mean response before injection was as follows: ■ = 10.37 ± 0.14 s; ▼ = 9.78 ± 0.18 s, ● = 10.5 ± 0.16 s and ▲ = 10.3 ± 0.18 s. (B) Anatomical mapping in successive frontal sections illustrating the distribution of some individual sites of microinjection in the PAG.