Neurobiology of Pain 3 (2018) 1–7

Contents lists available at ScienceDirect

Neurobiology of Pain

journal homepage: www.elsevier.com/locate/ynpai

Original Research

Dual effects of brain sparing opioid in newborn rats: Analgesia and hyperalgesia

Gong Kerui, Luc Jasmin*

Department of Oral and Maxillofacial Surgery, University of California San Francisco, San Francisco, CA, United States

ARTICLE INFO

This manuscript is dedicated to my son Léo-Paul Jasmin, born prematurely at 32 weeks on the 20th of December 2017.

Keywords:
Peripheral analgesia
Neonatal
Loperamide
Blood-brain barrier
Opiate induced hyperalgesia

ABSTRACT

Effective pain management in neonates without the unwanted central nervous system (CNS) side effects remains an unmet need. To circumvent these central effects we tested the peripherally acting (brain sparing) opioid agonist loperamide in neonate rats. Our results show that: 1) loperamide (1 mg/kg, s.c.) does not affect the thermal withdrawal latency in the normal hind paw while producing antinociception in all pups with an inflamed hind paw. 2) A dose of loperamide 5 times higher resulted in only 6.9 ng/mL of loperamide in the cerebrospinal fluid (CSF), confirming that loperamide minimally crosses the blood–brain barrier (BBB). 3) Unexpectedly, sustained administration of loperamide for 5 days resulted in a hyperalgesic behavior, as well as increased excitability (sensitization) of dorsal root ganglia (DRGs) and spinal nociceptive neurons. This indicates that opioid induced hyperalgesia (OIH) can be induced through the peripheral nervous system. Unless prevented, OIH could in itself be a limiting factor in the use of brain sparing opioids in the neonate.

Introduction

Unrelieved pain in the term and preterm neonate initiates maladaptive plasticity that can persist later in life (Schwaller and Fitzgerald, 2014; Walker et al., 2016). Opioids can prevent this plasticity while providing analgesia. There are concerns, however, that opioids have unwanted effects on the immature brain (Attarian et al., 2014; Beltran-Campos et al., 2015; de Graaf et al., 2011; Ferguson et al., 2012; Rozisky et al., 2011). For instance preemies who received opiates in the neonatal intensive care unit (NICU), can develop a smaller head-circumference, lower body weight, short-term memory impairments, and difficulty socializing (Attarian et al., 2014; Ferguson et al., 2012). In animal models, administrating opioids during the post-natal period leads to altered mu-opioid receptors (MORs) expression in the forebrain (Handelmann and Quirion, 1983), and increased pain behavior later in life (Rozisky et al., 2011). Given that opioids are effective analgesics for acute pain, a possible strategy is to use brain sparing (peripherally acting) opioids in the newborn. To explore this approach we chose the brain sparing MOR agonist loperamide (Guan et al., 2008; Kumar et al., 2012; Nozaki-Taguchi and Yaksh, 1999). Loperamide produces analgesia in adult models of inflammatory (Shannon and Lutz, 2002), cancer, and neuropathic pain (Chung et al., 2012; Guan et al., 2008) by acting on the peripheral opioid receptors (DeHaven-Hudkins et al., 1999; Guan et al., 2008). Accordingly, MORs in the periphery are critically involved in the analgesic effects of opioids (Taddese et al., 1995; Wang et al., 2010). Since there is a greater expression of MORs in primary sensory neurons during the first 2 post-natal weeks (Beland and Fitzgerald, 2001; Nandi et al., 2004), we postulated that newborns would be ideal candidates for loperamide induced antinociception. We tested loperamide in newborn rats, which are developmentally similar to premature humans (Romijn et al., 1991; Sengupta, 2013). We first assessed the effects of loperamide on the nociceptive withdrawal threshold in normal newborns, and then in newborns with an inflamed hind paw after a local carrageenan injection (Fehrenbacher et al., 2012). We then determined if loperamide crosses the blood–brain barrier (BBB) of the neonate rat. Finally, given that brain penetrant opioids can produce pro-nociceptive effects (Roeckel et al., 2017), we tested the effect of daily loperamide on the nociceptive threshold, the peripheral neuronal activity using patch clamp recordings, and the CNS activity using Fos immunochemistry.

Materials and methods

Experimental animals

Male and female Sprague-Dawley rats (Charles River Lab, USA), post-natal day 3 (P3) at the start of the experiment, were studied. Pups were kept with their littermates and mother in a dedicated room with

* Corresponding author at: Department of Oral and Maxillofacial Surgery, University of California San Francisco, 521 Parnassus Ave, Campus Box 0440, San Francisco, CA 94143, United States.
E-mail address: ljasmin@gmail.com (L. Jasmin).

https://doi.org/10.1016/j.ynpai.2018.01.001
Received 26 November 2017; Received in revised form 8 January 2018; Accepted 9 January 2018
Available online 10 January 2018

alternating 12 h of light-dark cycle. Food and water were available *ad libitum*. For each experimental group, 8–10 pups were used. No adverse effects of loperamide were observed during the experiment.

Ethics

Procedures for the maintenance and use of the experimental animals conformed to the regulations of UCSF Committees on Animal Research and were carried out in accordance with the guidelines of the NIH regulations on animal use and care (Publication 85–23, Revised 1996). The UCSF Institutional Animal Care and Use Committee approved the protocols for this study.

Experimental protocols

Loperamide and chemicals were purchased from Sigma-Aldrich unless noted otherwise.

For acute experiments, a single dose (1 mg/kg, s.c.) of loperamide 1 mg/mL or equal volume of vehicle (sterile 5% DMSO) was administered 30 min before carrageenan (1% in 0.9% saline, 20 µl, intradermal with a 30 ga needle) in the left hind paw. This preemptive analgesia mimics protocols promoting early interventions (drugs or others) in the NICU to prevent the long-term effects of untreated pain (Cignacco et al., 2009; Cruz et al., 2016; Laprairie et al., 2008).

Prior to the injection of carrageenan, but not prior to loperamide (Fig. 1A), rats were tested for the baseline thermal withdrawal latency (Hargreaves plantar test). In preliminary experiments we observed that loperamide 1 mg/kg did not increase the withdrawal latency in the Hargreaves test. We also found that decreasing the number of heat exposures in neonates minimizes the risk of stimulus induced paw sensitization. Rats were then retested at 5 min, 30 min, 1 h and 4 h after the carrageenan injection.

For chronic experiments, loperamide was administered once daily (1 mg/kg, s.c.) starting at P3 lasting until P7 (total of 5 days). Hind paw withdrawal latency to the heat stimulus was evaluated everyday starting on the first day prior to the initial dose of loperamide and then daily 6 h after each injection. This delay of 6 h, between the loperamide injection and the Hargreaves test, ensured that the nociceptive threshold was measured when the plasma levels of loperamide were high (He et al., 2000; Heel et al., 1978; Killinger et al., 1979; Miyazaki et al., 1979; Streel et al., 2005). Testing animals immediately prior to the daily injection of loperamide might have also showed hyperalgesia, whereas it could have been part of an early opioid withdrawal instead (Lee et al., 2011).

On each day, after pups were administered loperamide or tested, they were immediately returned to the dam. Precautions were taken to ensure that none of these newborns were rejected by their mother. During all manipulations and testing procedures, care was taken to maintain body temperature constant.

Control animals received the same volume of vehicle (sterile 5% DMSO) on the same schedule. After the last dose of loperamide or vehicle, pups (P7) were randomly injected with carrageenan or saline (20 µl, intradermal) in the left hind paw. Their lumbar spinal cord was collected and processed for Fos immunocytochemistry 3 h later.

Heat sensitivity (Hargreaves plantar test)

An investigator blind to the treatment groups performed the behavioral studies. Heat pain latency was measured using the Hargreaves plantar test device (Harvard apparatus, USA) (Cheah et al., 2017). Rats were placed into the test area 60 min prior to testing. The glass plate on which they were free to move was preheated to 30 °C to keep them comfortable. The withdrawal latency from a heat stimulus was measured 3 times for each hind paw, with a 5-min interval between individual measures. The mean value in seconds was used as the thermal nociceptive threshold. Although never reached, a cutoff of 20 s was used to prevent skin damage.

Biological fluid samples

To assess for possible penetration of loperamide in the CNS, we determined the concentration of loperamide in the CSF in P3 rats (n = 8) using mass spectroscopy (Rubelt et al., 2012). Serum levels were also determined by the same method. Based on a plasma half-life of 9–13 h (Doser et al., 1995; Killinger et al., 1979; Yu et al., 2004), a time to peak plasma concentration of 2.5 to 6 h (He et al., 2000; Heel et al., 1978; Killinger et al., 1979; Miyazaki et al., 1979; Streel et al., 2005), and a duration of action of up to 3 days (Heel et al., 1978), CSF and blood samples were acquired 6 h after a high dose of loperamide (5 mg/kg, s.c.).

CSF was obtained by puncture of the dura overlying the cisterna magna using an operating microscope and a pulled glass capillary pipette while the animals were under hypothermic anesthesia (Liu and Duff, 2008). Care was taken to make sure that the CSF was not contaminated by blood. Collection of blood was done by cardiac puncture into a 1.5 mL tube containing EGTA. The blood was spun down at 1500 g for 10 min in a refrigerated centrifuge. The supernatant (serum) was collected into a clean tube. CSF and serum samples were kept at −20 °C prior to analysis.

Serum and CSF loperamide levels were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using Agilent LC 1260-AB Sciex 5500 (binary pump, Agilent, USA). Each analyte was ionized using electrospray ionization in the negative mode and monitored by multiple reactions. The serum and CSF were prepared for LC-MS/MS analysis by solid phase extraction using Waters Oasis HLB cartridge (10 mg, 1 mL). Each cartridge was washed with 5 column

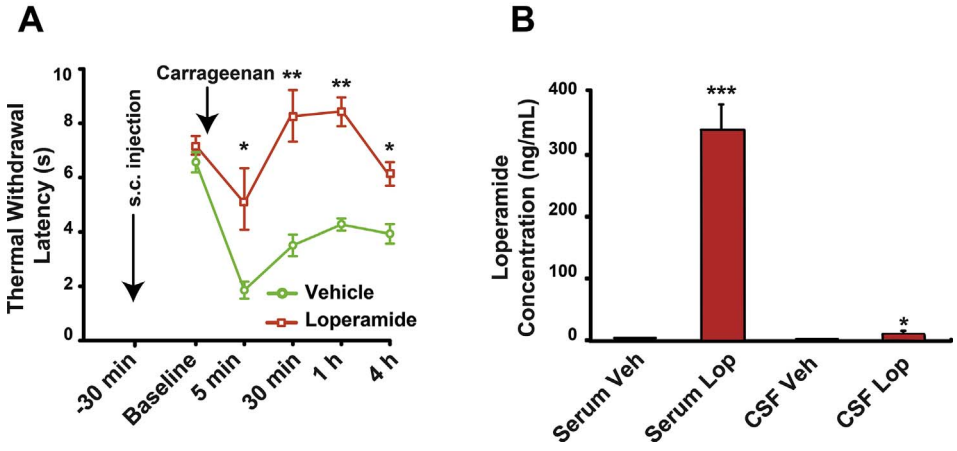

Fig. 1. Effect of a single dose of loperamide on thermal withdrawal latency, and systemic vs. central distribution. (A) Loperamide (1 mg/kg) or its vehicle were injected s.c. and 30 min later carrageenan (1% in 0.9% saline, 20 µl, intradermal) was injected in the left hind paw. The antinociceptive effect of loperamide was then monitored for the following 4 h (n = 10 for both groups) using the Hargreaves plantar test. P values are obtained after comparing Vehicle vs. Loperamide groups at each time point. (B) Concentrations of loperamide in the serum and CSF. Mass spectrometry showed that loperamide poorly penetrates the blood-brain barrier in neonates (P3). Six hours following 5 mg/kg, s.c., the concentration of loperamide in serum was 334 ng/mL, while in the CSF it was 6.9 ng/mL. P values are obtained by comparing the concentration of each treatment group with the 3 others. * P < .05, ** P < .01, *** P < .001.