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Ketoprofen and antinociception in hypo-oestrogenic Wistar rats fed on a high sucrose diet

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ABSTRACT

Non-steroidal anti-inflammatory drugs such as ketoprofen are the most commonly used analgesics for the treatment of pain. However, no studies have evaluated the analgesic response to ketoprofen in conditions of obesity. The aim of this study was to analyse the time course of nociceptive pain in Wistar rats with and without hypo-oestrogenism on a high sucrose diet and to compare the antinociceptive response using ketoprofen. Hypo-oestrogenic and naïve rats received a hyper caloric diet (30% sucrose) or water ad libitum for 17 weeks, the thermal nociception ("plantar test" method) and body weight were tested during this period. A biphasic response was observed: thermal latency decreased in the 4th week (hyperalgesia), while from 12th to 17th week, thermal latency increased (hypoalgesia) in hypo-oestrogenic rats fed with high sucrose diet compared with the hypo-oestrogenic control group. At 4th and 17th weeks, different doses of ketoprofen (1.8–100 mg/kg p.o.), were evaluated in all groups. The administration of ketoprofen at 4th and 17th weeks showed dose-dependent effects in the all groups; however, a greater pharmacological efficacy was observed in the 4th week in the hypo-oestrogenic animals that received sucrose. Nevertheless, in all the groups significantly diminish the antinociceptive effects in the 17th week. Our data showed that nociception was altered in the hypo-oestrogenic animals that were fed sucrose (hyperalgesia and hypoalgesia). Ketoprofen showed a dose-dependent antinociceptive effect at both time points. However, hypo-oestrogenism plus high-sucrose diet modifies the antinociceptive effect of ketoprofen.

1. Introduction

Pain is a condition that affects thousands of people in the world. However, it has been shown that individuals with increased body weight are more likely to have problems with pain (Marcus, 2004; Wilson et al., 2010). Controversies exist regarding the perception of pain in obese subjects. Some studies report a hyperalgesic state associated with obesity (Roane and Martin, 1990; Sugimoto et al., 2008; Buchenauer et al., 2009), while others have proposed the existence of hypoalgesic processes (Ramzan et al., 1993; Zhang et al., 1994; Sugimoto et al., 2008). Moreover, the most frequent cases that have been documented clinically in obese people involve back pain (Hitt et al., 2007; D'Arcy, 2011) and arthritis pain (Marcus, 2004), triggered mainly due to a mechanical effect on the joints by weight gain. Obesity has also been associated with other chronic pain conditions, such as migraine and headache (Goadsby et al., 2002; Rossi et al., 2013). For this reason, it is common for these patients to be treated with analgesic drugs. The most widespread drug therapy currently used to relieve nociceptive and inflammatory pain involves the non-steroidal anti-inflammatory analgesics (NSAIDs) and opioids (Ong et al., 2007). NSAIDs are the most widely prescribed drugs in clinical medicine; they are heterogeneous substances with varying nonsteroidal chemical structures (Laine, 2001). This group of drugs, which are indicated in the treatment of acute and chronic pain (Whiteside et al., 2004; Ong et al., 2007), is characterized by analgesic anti-inflammatory and antipyretic properties (Dworkin y Gitlin, 1991). Ketoprofen is a member of this group. As other NSAIDs, ketoprofen exerts its analgesic effect through at least three mechanisms of action, clearly identified as: 1) inhibition of prostaglandin synthesis (Avouac and Teule, 1988; Kubota et al., 1997), 2) interaction with the serotonergic system (Díaz-Reval et al., 2001, 2004) and 3) inhibition of proinflammatory cytokines (Choi et al., 2013). Preclinical studies have indicated that ketoprofen exhibits

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dose-dependent effects in different models of acute and inflammatory pain (Díaz-Reval et al., 2001, 2004; Aguilar-Carrasco et al., 2014). However, no studies have evaluated the analgesic response of ketoprofen in conditions where there is an increase in body weight; therefore, the antinociceptive effect it may have under these conditions is unknown. The effects may differ because pathological conditions can change the activity of drugs, mainly through pharmacokinetic and/or pharmacodynamic alterations (Lloret et al., 2009).

Therefore, the objectives of this study were to evaluate nociception in hypo-oestrogenic and naïve female Wistar rats under conditions of increasing weight up to a state of obesity achieved through the provision of a high sucrose diet (30% in drinking water) and to analyse the antinociceptive response to ketoprofen.

2. Materials and methods

2.1. Animals and housing conditions

Female Wistar rats [Crl(WI)fBR] weighing 180–200 g at the time of surgery were used in this study. All animals were obtained from the animal breeding facility of the Centre of Investigation and Advanced Studies (Cinvestav, Sede Sur). The animals were housed under standardized conditions in a room on a 12 h light/dark cycle with food and water available ad libitum before treatment. All experimental procedures were approved by the Local Ethics Committee for the Management of Laboratory Animals of the Department of Pharmacobiology of Cinvestav, Sede Sur, following the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmermann, 1983) and the Official Mexican Norm (NOM-062-ZOO-1999). All tests were performed during the light phase. The number of experimental animals was kept to a minimum, and the rats were killed immediately after the experiment by CO_2 overdose.

2.2. Compounds

Refined sucrose was obtained commercially from Supplies Ben-Hill, S. A. de C. V. (Mexico City, Mexico). The sugar was dissolved in water to 30% (wt/vol). Ketoprofen was obtained from Sigma Chemical Company (St. Louis, MO, USA). Ketoprofen was suspended in 0.5% carboxymethylcellulose and was administered orally in an application volume of 4 ml/kg body weight. The doses mentioned in the text refer to the salts of the substances used.

2.3. Surgical technique (Ovariectomy)

Briefly, the ovariectomy consisted of the following procedures. All animals were anaesthetized by an intraperitoneal (i.p.) injection of 50 mg/kg ketamine and 10 mg/kg xylazine mixture for bilateral removal of the ovaries. Briefly, the abdominal and pelvic areas of the animals were shaved and cleaned. A longitudinal incision of approximately 1.5 cm was made, the skin was separated from the muscle, and a second incision of approximately 0.5 cm was made in the muscle to exteriorize the ovaries. The fallopian tubes were ligated and cut below the ligature. After the excision, the incision was sutured. This surgery caused a state of hypo-oestrogenism in experimental animals. The ovariectomized female rat model has been commonly used as a direct cause of increased body weight (Stubbins et al., 2012; Da Silva et al., 2014) and as a model for menopause in humans (Diaz Brinton, 2012). All procedures were carried out under aseptic conditions.

2.4. Measurement of antinociceptive activity

To avoid additional stress, the rats were allowed to acclimate to the testing site until exploratory behaviour diminished for at least 10 min before stimulation was initiated. Responses to thermal nociception were evaluated using the Hargreaves method (UGO-BASILE, Varese, Italy) (Hargreaves et al., 1988). Briefly, a radiant heat source with a locator light was positioned under the plantar surface and the latency to withdrawal the right hind paw was recorded for each animal. A resting period of 5 min followed to avoid any sensitization. The intensity of the lamp was set at 60 Hz, and a cut-off time of 30 s (s) was determined necessary to avoid tissue damage. The light beam was directed on the plantar surface of the hind paw until the animal responded, or when it came to cutting time (30 s), the lamp was turned off, whichever occurred first. The latency of the paw withdrawal from the incident light was recorded with a built-in timer, which displayed reaction time in 0.01 s increments. The latency of withdrawal was defined as the time between the initiation of the light and the moment at which the animal withdrew from the tip of the light quickly, either generating a limb-licking behaviour or jumping away from the heat source. Three readings were made per animal. Data are expressed as the withdrawal latency and are measured in seconds.

2.5. Experimental design

On the 15th postoperative day (ovariectomy), the time course of the thermal latency was initiated. Female Wistar rats weighing 250–270 g each were randomized into four groups (n=6) as follows: hypo-oestrogenic-control (Ov-Ctrl), hypo-oestrogenic-sucrose (Ov-Suc) (both were ovariectomized), naïve-control (Naïve-Ctrl) and naïve-sucrose (Naïve-Suc) (both were non-operated). Before the start of the diet, baseline thermal threshold responses of all groups were measured at time 0 (immediately after receiving the respective diet). During the 17-weeks treatment, all groups were given free access to food (pellets of LabDiet 5008). Additionally, sucrose fed rats received 30% commercially refined sucrose (wt/vol) in drinking water given ad libitum. In contrast, the control group received filtered drinking water ad libitum. During this period, thermal nociception was assessed weekly using Hargreaves method (UGO-BASILE, Varese, Italy) (Hargreaves et al., 1988), and body weight measurements were taken.

The effects of the acute administration of a drug (ketoprofen) on response thresholds to thermal stimuli were tested at week 4 and week 17 post-treatment with sucrose or water ad libitum in ovariectomized rats. Baseline thermal nociception was assessed before pharmacological testing. The experimental protocol consisted of two sets of experimental groups in which the antinociceptive effects produced by ketoprofen, given individually, were studied with the corresponding vehicle (0.5% carboxymethylcellulose). In the first set of experimental groups, each dose of ketoprofen (1.8, 5.6, 10, 17.8 or 31.6 mg/kg, p.o.) was given in a volume of 4 ml/kg to six hypo-oestrogenic and Naïve rats (that had received the respective diet for 4 weeks) to obtain the dose-response curves (DRC). In the second set, hypo-oestrogenic and Naïve animals that were administered either sugar-free or sucrose diets for 17 weeks were treated at the end of this period with ketoprofen at different doses (10, 31.6 or 100 mg/kg p.o.) along with the corresponding vehicle to test for antinociceptive effects. The rats were tested every 30 min in the 240 min (4 h) post-administration period.

2.6. Statistical analyses

All data are expressed as the mean ± S.E.M. and were checked for normality using the Shapiro-Wilk test. The thermal nociceptive