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Research report

Activation of the trigeminal α_2-adrenoceptor produces sex-specific, estrogen dependent thermal antinociception and antihyperalgesia using an operant pain assay in the rat

Subodh Nag*, Sukhbir S. Mokha

Department of Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN 37208, United States

HIGHLIGHTS

- Trigeminal α_2-adrenoceptor activation produces analgesia using an operant pain test.
- Estrogen abolishes α_2-adrenoceptor analgesia and antihyperalgesia in females.
- Intracisternal administration of clonidine does not produce hyperphagia.

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ABSTRACT

Higher prevalence of several pain disorders in women and sexual dimorphism in G-protein coupled receptor-induced analgesia has been reported. We have previously shown that α_2-adrenoceptor-induced antinociception is sex-specific and attenuated by estrogen in the female rat. However, this evidence was obtained using reflexive withdrawal-based nociceptive assays conducted on restrained animals that may not involve cerebral processing. Hence, we evaluated whether activation of the trigeminal α_2-adrenoceptor produces sex-specific antinociceptive and antihyperalgesic effects in the orofacial region of the rat using a reward conflict-based operant paradigm in which animals must tolerate nociceptive thermal stimulation to be rewarded. Male and ovariectomized (OVX) Sprague-Dawley rats were implanted intracisternally with a PE10 cannula for drug injections. A group of OVX rats (OVX+E) was administered subcutaneously with estradiol 48 h before the test. Effect of clonidine, an α_2-adrenoceptor agonist, was determined on the operant pain assay using a fully automated Orofacial Pain Assessment Device. Number of spout licks, thermode contacts, and amount of reward intake were automatically recorded by the ANY-maze software. Using acute pain modeling, clonidine produced a dose-dependent increase in all three parameters in male and OVX groups, however, it was ineffective in the OVX+E group. Similarly, using inflammatory pain modeling, clonidine significantly increased these parameters in carrageenan-treated male and OVX groups but not in the OVX+E group. Thus, α_2-adrenoceptor activation produces sex-specific antinociception and antihyperalgesia and estrogen attenuates these effects in female rats using an operant pain assay. These findings may help the discovery of effective analgesics for each sex.

1. Introduction

Sex-related differences in pain have been reported with women exhibiting a higher prevalence of several pain disorders, e.g. temporomandibular joint disorder, migraines, irritable bowel syndrome and fibromyalgia as well as some women-specific pain conditions viz., endometriosis, vulvodynia and menstrual pain [1–9]. Relatively high levels of gonadal hormones present in women during the reproductive years have been implicated for enhanced pain perception [10–12]. Further, sexual dimorphism in the G-protein coupled receptor (GPCR)-mediated analgesia have been reported in clinical [13–15] and animal studies [5,16–27]. In this regard, we have previously shown that antinociception induced by activation of the α_2-adrenoceptor (a GPCR) is sex-specific, attenuated by estrogen in the female and requires testosterone in male rats [20–23]. However, experimental evidence thus far has been predominantly obtained from studies conducted using traditional, reflexive withdrawal-based pain assays in often restrained animals that may not involve cerebral processing. With the advent of the recent orofacial nociceptive assay, based on the reward-conflict,

* Corresponding author at: Department of Neuroscience and Pharmacology, Meharry Medical College, 1005 D.B. Todd Blvd, Nashville, TN 37208, United States.
E-mail address: snag@mmc.edu (S. Nag).

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operant paradigm in which animals must tolerate nociceptive thermal stimulation in the orofacial region to obtain access to the reward, it has become feasible to access nociception in a manner that better approximates complex, sensory and emotional human pain state in experimental rodents [28,29] using the Orofacial Pain Assessment Device (OPAD). Hence, we investigated whether intracisternal administration of clonidine (an α_2-adrenoceptor agonist) produces antinociception in the orofacial region of the rat using the OPAD. In addition to a test for acute thermal nociception, an inflammation-induced thermal hyperalgesia model using carrageenan injection in the orofacial region was also employed since it mimics an important, persistent pain and hyperalgesia condition commonly observed in patients with pain disorders as well as following injuries and surgical procedures. Moreover, we investigated whether estrogen attenuates α_2-adrenoceptor-mediated antinociception and antihyperalgesia using this operant assay of pain.

2. Materials and methods

2.1. Subjects

Sprague–Dawley male and ovariectomized (OVX) female rats (225–249 g; Harlan Sprague-Dawley, Inc., Indianapolis, IN) were housed in the animal care facility at Meharry Medical College certified by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) under a 12-h light/dark cycle (lights on: 7:00 AM) and had free access to food and water. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Meharry Medical College and conformed to the guidelines established by the National Research Council Guide for the Care and Use of Laboratory Animals and the International Association for the Study of Pain (IASP).

2.2. Intracisternal cannulation

As previously described [21,22,30] animals were anesthetized using ketamine and xylazine anesthesia (80 and 4 mg/kg respectively; i.p.), their heads were shaved and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The skin above the head/neck region was incised, the atlantooccipital membrane was removed and the dura was exposed. A tiny opening was made in the dura using a 25 gauge sterile needle and the tip of a PE-10 cannula (dead volume 7 μl) was inserted approximately 1.5 mm through the opening. The cannula was then secured to the skull with dental cement and the wound closed using 9 mm Autoclips (BD, Franklin, NJ). Animals were kept on a heating pad (36 °C) till they regained consciousness and were then returned to their home cages for recovery.

2.3. Orofacial thermal pain assay based on operant paradigm

The newly developed, fully automated OPAD (Stoelting, Wood Dale, IL) was used in which animals were presented with a choice of experiencing aversive temperatures to gain access to a liquid reward (1:3 diluted, sweetened condensed milk solution in water [28]. The access to the spout of reward containing bottle was via a thermode-lined opening with which animal's cheeks made contact as it consumed the reward. After a postoperative recovery period of 5–7 days, animals were food deprived overnight and were first trained to consume ~10 g of reward in one, 30 min session. Three to five training sessions (one/day) were administered. Thermode temperature was set at an ambient 24.8 °C during this training. On the test day (following the last training day), animal's cheek region on both sides of the head was carefully shaved using hair clippers under 5% isoflurane anesthesia combined with O_2 using a vaporizer (VETEQUIP, Pleasanton, CA). Thermode temperature was raised to 48 °C for acute pain testing and 45 °C to test thermal hyperalgesia following carrageenan injection. The OPAD was under complete control of ANY-maze software (Stoelting) running on a PC that automatically regulated the thermode temperature, recorded number of licks to the spout (licks) and contacts to the thermodes (contacts), and the amount of reward consumed (reward intake), and terminated trial/test at the completion of 30-min time period. A ratio between number of licks to the spout and thermode contacts was also calculated and analyzed (licks/contact).

2.4. Carrageenan injection

As described [28], vehicle (sterile saline) or lambda carrageenan (4%; 0.15 ml; Sigma Chemical, St. Louis, MO) was injected subcutaneously (s.c.), bilaterally into the mid cheek region using a 27G needle attached to a sterile, disposable 1 ml syringe, under 5% isoflurane anesthesia in trained rats. Cheek region was shaved at the same time. Operant pain testing was conducted at the thermode temperature of 45 °C to access thermal hyperalgesia three hours subsequent to the carrageenan injection.

2.5. Estradiol replacement

A group of OVX rats (OVX+E) was administered 17β-estradiol 3-benzoate (100 μg/100 μl; s.c.) 48 h before the operant pain test. This protocol has been used previously in neuroendocrinology [31,32] and in the pain field by us [20,21,24,26] and others [33,34]. Further, this dose of estradiol has been shown to most reliably induce lordosis behavior in rats [35] and we have previously shown that it yields serum-estradiol levels and vaginal smear cytology comparable to that at proestrous stage of the estrous cycle after 48 h – the time point for behavioral testing in this study [21,24]. OVX group was given the vehicle (sesame oil; s.c.). Normally cycling females at pro- and diestrous stages were not included this in study since estrous cycle is disrupted for prolonged periods following surgery and implantation of cannulae under anesthesia. The unpredictable time period required for the restoration of estrous cycle would further disrupt training/testing protocol due to overtraining and/or uneven time interval between cannula implantation, training and testing.

2.6. Drugs

All drugs were obtained from Sigma–Aldrich (St. Louis, MO) and injected intracisternally. Clonidine (0, 0.875 or 1.75 μg/5 μl) was injected 5 min before the test in separate groups (n = 6/group for acute assay and 5/group for hyperalgesia assay). Yohimbine (30 μg/15 μl), an α_2-adrenoceptor antagonist was injected 5 min before clonidine. We have used these drug doses successfully in our previous studies employing NMDA-induced scratching behavior and thermal head-withdrawal assays [21]. Previously used higher doses of clonidine (3.5 and 7 μg) produced sedation in our pilot experiments using OPAD and were excluded. All experiments were conducted between 9 AM – 2 PM. Each rat was tested only once and all rats were euthanized by an intraperitoneal injection of Beuthanasia (150 mg/kg; Schering-Plough, Kenilworth, NJ) or CO_2 at the end of the experiment. All efforts were made to minimize stress to the animals and the number of animals used.

2.7. Data analysis

Data presented in Figs. 1 and 2 were analyzed by ANOVA and that in Fig. 3 by *t*-test using IBM SPSS (IBM Corp., Armonk, NY, USA) with appropriate between group factor and dependent variables. Fisher's post-hoc test was used for intergroup comparisons where ANOVA yielded a significant main effect. Significance level was set