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Infusions of naloxone into the medial preoptic area, ventromedial nucleus of the hypothalamus, and amygdala block conditioned place preference induced by paced mating behavior

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ABSTRACT

Paced mating induces positive affect as revealed by conditioned place preference (CPP) in female rats. It has been suggested that endogenous opioids are involved in the generation of this positive affect since systemic administration of the opioid antagonist naloxone blocks mating-induced CPP. Several brain structures, including the medial preoptic area (mPOA), the ventromedial nucleus of the hypothalamus (VMH), the amygdala (Me), and the nucleus accumbens (Acb) have been implicated in the control of female sexual behavior. However, it is not known if these structures also participate in the positive affect produced by paced mating. To this end we determined the effects of intracranial administration of naloxone methiodide into the mPOA, VMH, Me and Acb on conditioned place preference induced by paced mating in female rats. Regardless of the site of infusion 5 μg of naloxone did not affect any of the sexual behavior parameters measured during copulation. When CPP was evaluated, the groups infused with naloxone into the mPOA, the VMH, and the Me before each conditioning session did not develop place preference. Only the group infused with naloxone in the Acb and the control groups did so. These results demonstrate that opioid receptors within the mPOA, VMH and Me are necessary for the rewarding aspects of paced mating. We suggest that the Me and VMH are important for the transmission of sensory information produced by copulation while the mPOA is the site where the positive affect is originated.

Introduction

The conditioned place preference (CPP) procedure was developed many years ago for studying the affective consequences of drugs (Beach, 1957a,b; Rossi and Reid, 1976). Essentially, it consists of the establishment of an association between a potential affective state and environmental cues through classical conditioning. After conditioning, the cues associated with positive affect activate approach behaviors superior to those activated by simultaneously present, neutral cues (see Spiteri et al., 2000, for a detailed discussion). In addition to studies of drug-induced reward, the CPP procedure has been extensively used in studies of positive affect produced by natural rewards like eating, drinking, copulation and wheel running (reviewed in Tzschentke, 1998).

We have previously shown that females that are allowed to control the rate of sexual stimulation during copulation (paced mating) develop a CPP (Garcia et al., 2004; Martinez and Paredes, 2001; Paredes and Alonso, 1997; Paredes and Vazquez, 1999). The establishment of this CPP is blocked by systemic administration of the opioid antagonist naloxone (Paredes and Martinez, 2001) showing that activation of opioid receptors is necessary for mating-induced CPP. Likewise, females allowed to copulate with scented male rats in the pacing procedure and with differently scented males in a non-paced procedure prefer to copulate with a pacing-scented male over a non-pacing scented male when both are available simultaneously (Coria-Avila et al., 2005). This preference is abolished in females treated with naloxone during the acquisition of the association between a particular scent and a particular mating situation (Coria-Avila et al., 2008). It was proposed that naloxone blocked the superior reward value of paced mating, a proposal entirely in agreement with the place preference studies mentioned earlier.

There is little understanding of the neural events that transduce mating stimulation into the affective state induced by paced mating. Given the complex interplay of different sensorimotor and motivational stimuli engaged during mating it seems clear that several brain structures are involved. Indeed, studies of FOS activation after paced mating (Erskine and Hanrahan, 1997) or after exposure to stimuli associated with paced mating (Coria-Avila and Pfaus, 2007) have shown that the amygdala (Me), the ventromedial nucleus of the hypothalamus (VMH), and the medial preoptic area (mPOA) are activated. It is likely that the affective consequences of mating originate in or can be mediated by one or several of these structures.

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The basis for the positive affect produced by paced mating must be the sensory stimulation provided by the male during sexual interaction. Control procedures have eliminated the simple exposure to the male as sufficient for sexual positive affect (Paredes and Alonso, 1997; Paredes and Vazquez, 1999), and it is most likely that the vaginocervical stimulation obtained during mating is crucial. Indeed, vaginocervical stimulation provided by a syringe plunger has been reported to be enough for producing place preference (Meerts and Clark, 2007; See also Coria-Avila et al., 2008). The brain structures activated by vaginocervical stimulation (VCS) overlap with those activated by paced mating (Erskine and Hanrahan, 1997; Pfaus et al., 2006). It is also known that vaginocervical stimulation produces analgesia (Adler et al., 1977; Komisaruk and Whipple, 1995), and this analgesia is at least partly blocked by systemic administration of an opiate antagonist (see Komisaruk and Whipple, 2000). These observations suggest that opioids are released in response to stimulation of the vagina/cervix. The fact that an opioid antagonist blocks paced mating-induced CPP further reinforces the notion of opioid release in response to the genital stimulation obtained during copulation. Thus, data from studies involving sexual reward and from studies of mating-related analgesia coincide in suggesting that opioids are released during the course of copulation in female rats.

The purpose of the present experiment was to determine at which brain sites a potential mating-induced opioid release may produce positive affect. To that end, quaternary naloxone was infused before paced mating into brain areas activated by female sexual behavior as well as by VCS and where opioid receptors are abundant (Acosta-Martinez and Etgen, 2002a; Acosta-Martinez and Etgen, 2002b; Desjardins et al., 1990; Pierce and Wessendorf, 2000; Simon and Jacob, 1994; Sinchak et al., 2007; Sinchak and Micevych, 2003). These areas are the amygdala (Me), the ventromedial nucleus of the hypothalamus (VMH), and the medial preoptic area (mPOA). If naloxone would be able to block paced mating-induced CPP after infusion into any or several of these structures it could be concluded that opioid receptors within that/these structures indeed are important for sexual positive affect. Since the nucleus accumbens (Acb) has been associated with both drug-induced and natural reward in many studies (Kalivas et al., 2006; Van Ree et al., 2000; Ward et al., 2006) one group of females was infused with naloxone into that structure.

Method

Animals

Seventy-six sexually naive female Wistar rats from a local colony (280 to 350 g) were used in all experiments. All animals were maintained under a reversed light/dark cycle (12/12 h) at constant room temperature (approximately 22 °C) with free access to water and commercial rodent pellets (LabDiet, Nutrition International, Brentwood, MO). For the mating tests, intact sexually experienced male Wistar rats (320g to 480 g) were used as partners. After implantation of intracerebral cannulae females were housed individually in plastic cages to prevent other animals from biting the cannula cap. All the experiments were carried out in accordance with the "Reglamento de la Ley General de Salud en Materia de Investigación para la Salud" of the Mexican Health Ministry which follows NIH guidelines for care and use of animals in research.

Apparatus

Pacing chamber

The pacing chamber consists of an acrylic box (40 cm×60 cm×40 cm) with wood shavings on the floor. It is divided into halves by a partition with a small hole (4 cm diameter) on the bottom through which only the female can move from one side to the other. The hole is too small for the male to get through.

Place preference cage

Three-compartment boxes made of wood are used. The lateral compartments (23 cm×37 cm×32 cm) are connected to a central compartment (22cm×24 cm×32 cm). One of the lateral compartments is painted white, and the floor is covered with fresh wood shavings. The opposite lateral compartment is painted black and moistened with a 2% solution of glacial acetic acid immediately before an animal is placed in it. The middle compartment is painted gray and communicates with the lateral compartments through a 10×10 cm sliding door on each side. The front side of the middle compartment is made of fine wire mesh, which allows observation of the animal inside the cage. The place preference cages are located in a room illuminated with dim white light.

Surgery

Females were anesthetized with a combination anesthetic (1 ml/kg body weight) containing ketamine (95 mg/ml) and xylazine (12 mg/ml) administered ip. Then they were bilaterally implanted with 22-gauge stainless steel guide cannulae (Plastics One Roanoke, VA) aimed at either the mPOA (coordinates: 0.2 mm posterior to Bregma, 0.5 mm lateral to the midline, and 7.1 mm ventral to the surface of the skull); the VMH (coordinates: 2.4 mm posterior to Bregma, 1.5 mm lateral to the midline and 7.5 mm ventral to the surface of the skull); the Me (coordinates: 3.1 mm posterior to Bregma, 3.6 mm lateral to the middle line, and 7.5 mm ventral to the surface of the skull); or the Acb (coordinates: 1.7 mm anterior to Bregma, 1.5 mm lateral to the midline and 6.2 mm ventral to the surface of the skull). All the cannulae were implanted with the skull flat and were fixed to the skull with two jewelry screws and dental cement. Each cannula was covered with a dust cap immediately after implantation. Coordinates were based on the rat brain atlas of Paxinos and Watson (2005). Immediately After implantation, the females were ovariectomized. After surgery all subjects received an intramuscular injection of penicillin G procaine (Virbac S.A. de C.V. México) to prevent infection.

Hormone treatment

After surgery all the animals were allowed to recover for five days. Then, all females received 25 μg of estradiol benzoate (EB) 48 h and 1 mg of progesterone (P) 4 h before the sexual behavior tests. Hormones were previously dissolved in corn oil vehicle and subcutaneously injected in a volume of 0.2 ml per rat. They were purchased from Sigma (St, Louis, MO).

Sexual behavior test

In each test, a stimulus male was placed on one side of the partition 2 min before the female was introduced on the other side. During each session the following behaviors were recorded: mount and intromission latencies (time from the introduction of the female into the arena until the first mount or intromission, respectively), and ejaculation latency (time between the first intromission and ejaculation), as well as number of mounts and intromissions. The following parameters were also calculated: lordosis quotient [LQ; (number of lordosis/(number of mounts+number of intromissions)×100], lordosis reflex score (LS); calculated for each lordosis response ranked in intensity from 0 to 2 according to the extent of dorsiflexion observed, modified from (Hardy and DeBold, 1972). In order to obtain the mean lordosis intensity (MLI), the sum of lordosis points was divided by the number of mounts plus intromissions received. The exits from the male chamber (in a 5 s interval) after a copulatory event were determined and expressed as percentage of mounts (% EM) or intromissions (% EI). The test ended when the female had received 15 intromissions with or without ejaculation.