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Glutamatergic transmission is involved in the long lasting sexual inhibition of sexually exhausted male rats

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ABSTRACT

Copulation to satiation induces a series of enduring physiological changes in male rats, with the appearance of a long lasting sexual inhibitory period as the most conspicuous, that are suggestive of the occurrence of neuroplastic changes. Copulation is a natural reward activating the mesocorticolimbic circuit and inducing nucleus accumbens dopamine release. The repeated activation of this system by drug rewards induces neuroplastic changes involving both dopamine and glutamate transmission. We hypothesized that repeated activation of the mesocorticolimbic circuit during copulation to satiation might also activate these neurotransmitter systems. The objective of the present work was to establish the possible participation of glutamate transmission in sexual satiety. To this aim we tested if the systemic injection of specific glutamate receptor antagonists of the NMDA, AMPA and mGluR5 receptor subtypes would reverse the sexual inhibitory state characteristic of sexually satiated rats. Results showed that systemic administration of low doses of the three glutamate receptor antagonists reversed sexual exhaustion evidencing a role for glutamate in the maintenance of the sexual inhibition that follows copulation to satiation, with the participation of NMDA, AMPA and mGluR5 receptors. These glutamate receptor subtypes have been associated to the neuroplastic changes resulting from repeated activation of the mesocorticolimbic circuit by drug rewards, a phenomenon that might also result from its activation by continued copulation.

1. Introduction

Sexual satiation is a phenomenon consisting of a long lasting sexual behavior inhibition (at least 72 h) that appears after repeated ejaculation during ad libitum copulation of a sexually experienced male rat with a sexually receptive female (Beach and Jordan, 1956). Twenty-four hours after copulation to exhaustion, male rats express sexual satiation in two different manners: either they do not respond to the presence of a receptive female or they copulate until attaining a first ejaculation, after which males do not resume copulation (Rodríguez-Manzo and Fernández-Guasti, 1994). This inhibitory state is long lasting, since although all animals show sexual activity after the initial 72 h, their ejaculatory capacity is limited. It is only after a 15 day rest period that satiated males show a complete recovery of their sexual capacity (Rodríguez-Manzo et al., 2011). The long lasting nature of the phenomenon suggests the occurrence of transient neuroplastic changes as a result of intense copulation. In favor of this idea, sexually exhausted male rats exhibit additional physiological changes when compared to sexually experienced, non-exhausted animals. Among the most conspicuous we can mention that sexually satiated animals exhibit a generalized hypersensitivity to drug actions (Rodríguez-Manzo et al., 2011) and that the sexual stimulatory effects produced by electrical stimulation of specific brain regions in sexually experienced animals, get lost once rats become sexually exhausted (Rodríguez-Manzo and Pellicer, 2007, 2010; Rodríguez-Manzo et al., 2000). The sexual inhibitory state that characterizes sexually satiated rats can be reversed by pharmacological treatment with specific receptor agonists or antagonists targeting receptors of diverse neurotransmitter systems. All these drugs, however, appear to interact with the dopaminergic system to reverse satiation. Thus, the α2 receptor antagonist yohimbine, the 5-HT1A receptor agonist 8-OH-DPAT and the μ and δ opioid receptor antagonist naloxone lose their ability to reverse satiation in brain noradrenaline-depleted rats (Rodríguez-Manzo & Fernández-Guasti, 1995). Besides, noradrenaline interacts with dopamine to reverse sexual exhaustion (Rodríguez-Manzo, 1999), while general enhancement of dopaminergic transmission with apomorphine and the specific activation of D1-like receptors reverse satiation (Guadarrama-Bazante et al., 2014). Finally, the pharmacological manipulation of two modulating systems of the dopaminergic tone at the mesolimbic circuit, endogenous opioids and endocannabinoids, can block the establishment of and reverse the already established sexual inhibition that characterizes sexual satiation (Canseco-Alba and Rodríguez-Manzo, 2014; Garduño-Gutiérrez et al., 2013a). Together these data point to dopaminergic transmission as a final common pathway for the reversal of this sexual inhibitory state.

Sexual behavior is a natural reward activating the dopaminergic mesocorticolimbic system (MLS) (Balfour et al., 2004; Kelley and

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Berridge, 2002) and producing increases in dopamine release within the nucleus accumbens (NAcc) (Damsma et al., 1992; Pfaus et al., 1990). Increased dopamine release in this brain region is also produced by abused psychostimulants, such as cocaine and amphetamine (Di Chiara and Imperato, 1988) and it has been proposed that these dopaminergic elevations induce transient increases in glutamate transmission as part of a cascade of events facilitating the occurrence of neuroplastic changes (Kalivas, 2004). Repeated activation of the MLS by drug rewards induces neuroplastic changes mediated both by the dopaminergic and glutamatergic systems (Kalivas et al., 2009; Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000). Besides, interactions between glutamate and dopamine have been involved in the modulation of motivation and reward (Choi et al., 2005), as well as in synaptic plasticity (Harnett et al., 2009; Scott and Aperia, 2009).

During copulation to exhaustion the MLS is constantly activated as evidenced by the increases in NAcc dopamine release recorded along the satiation process (Fiorino et al., 1997). Thus, this continued MLS stimulation could induce neuroplastic changes underlying the behavioral changes observed in sexually satiated rats, including the establishment of the long lasting sexual inhibition. In favor of a role of the MLS in the satiation phenomenon is the fact that the μ and δ opioid receptor antagonist naltrexone, directly infused into the ventral tegmental area (VTA), reverses satiation as it does when systemically administered (Garduño-Gutiérrez et al., 2013b).

In relation to MLS glutamatergic transmission, it has been reported that sexual experience causes long-lasting changes in ionotropic glutamate receptor expression and function in the NAcc (Pitchers et al., 2012).

Glutamate exerts its actions through the activation of different ionotropic and metabotropic receptors. Among them, the N-methyl-D-aspartate (NMDA) (Karler et al., 1989), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Pierce et al., 1996) and the metabotropic glutamate 5 receptor (mGluR5) (Chiamulera et al., 2001) have been associated to the neuroplastic changes resulting from repeated activation of the MLS by drug rewards. Thus, we hypothesized that these glutamatergic receptors might participate in putative neuroplastic changes underlying the long lasting sexual inhibitory phenomenon characteristic of sexual satiation. On these bases, the purpose of the present study was to investigate the possible involvement of glutamatergic transmission in the maintenance of the long lasting sexual behavior inhibition resulting from copulation to satiety. To this aim, we tested if the systemic injection of specific NMDA, AMPA and mGluR5 glutamate receptor antagonists would reverse the sexual inhibitory state of sexually exhausted male rats.

2. Materials and methods

2.1. Animals

181 sexually experienced adult male Wistar rats (250–300 g body weight) were housed, eight per cage, in a room under inverted light: dark cycle conditions (12 h light/12 h dark; lights on at 22:00 h) with free access to commercial rat chow and tap water. For the selection of sexually experienced males, rats were subjected to five independent sexual behavior tests, run every other day, and those animals showing ejaculation latencies shorter than 15 min, in at least three of these tests, were selected for the study. All experimental procedures were approved by the Institutional Committee of Ethics on Animal Experimentation which follows the regulations established in the Mexican official norm for the use and care of laboratory animals NOM-062-ZOO-1999.

Receptive female rats were used as stimuli. Receptivity was induced in intact female rats by the sequential subcutaneous injection of estradiol benzoate (4 μg/rat) followed 44 h later by progesterone (2 mg/rat).

2.2. Sexual behavior observations

Sexual behavior tests were conducted under dim red light, 2 h after the onset of darkness and 4 h after progesterone injection to the females. Male rats were introduced into a cylindrical polycarbonate arena (40 cm diameter, 60 cm height) and a 5 min adaptation period was allowed before introducing a single receptive female. The proportion of sexually exhausted males in each group that showed the different sexual behavior responses, i.e. mount, intromission, ejaculation and copulation resumption after ejaculation was registered. In those animals attaining ejaculation the following sexual behavior parameters were determined: a) intromission latency (IL): time from the introduction of the female into the observation cage to the occurrence of the first intromission; b) number of mounts (M) preceding ejaculation; c) number of intromissions (I) preceding ejaculation; d) ejaculation latency (EL): time from the first intromission to ejaculation and e) post-ejaculatory interval (PEI): time from ejaculation to the first intromission of the next copulatory series.

2.3. Sexual exhaustion paradigm

Sexually experienced male rats were subjected to a 4 h session of ad libitum copulation with a single stimulus female to render them sexually exhausted. This time period has been proved to be sufficient for all male rats to achieve sexual exhaustion, independently of their individual ejaculatory capacity (Rodríguez-Manzo and Fernández-Guasti, 1994). Twenty four hours later these same animals were randomly assigned to the distinct pharmacological treatments and tested for sexual behavior with a new receptive female. This last observation was ended ensuing one of the following criteria: a) a period of 30 min without showing sexual behavior; b) 30 min from the first intromission without achieving ejaculation; c) 30 min after ejaculation; or d) immediately after the first intromission of a second ejaculatory series.

2.4. Spontaneous ambulatory behavior

In order to discard motor alterations that could have interfered with sexual behavior display, all rats were tested for spontaneous ambulation immediately after the sexual behavior tests that followed the pharmacological treatments. Ambulatory activity was recorded in an acrylic box measuring 43 × 36 × 19 cm, with the bottom divided into 12 squares (12 cm × 12 cm). The animals were placed into the cage and the number of times they crossed from one square to another was recorded during a 5 min period. The cage was carefully cleaned between tests.

2.5. Drugs

The non-competitive NMDA receptor antagonist ketamine hydrochloride [(+/−)-2-(chlorophenyl)-2-(methylamino)-cyclohexan-1-one)] (Probiomed, Mexico), the AMPA receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and the mGluR5 glutamate receptor antagonist MPEP (6-methyl-2-(phenylethynyl)-pyridine) (Sigma Chem. Co., St. Louis, Mo, USA) were all dissolved in saline solution and intraperitoneally (i.p.) injected in a volume of 1 ml/kg.

2.6. Statistical analyses

Statistical differences in the proportion of sexually exhausted animals showing mounts, intromissions, ejaculation and resuming copulation after ejaculation were established by means of the Fisher *F*-test. Since a very low number of the sexually satiated animals treated with vehicle ejaculate during the 24 h test, the sexual behavior parameters of animals in which sexual exhaustion was reversed by the pharmacological treatments were compared with those of an independent