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Regulation of sexual odor preference by sex steroids in the posterodorsal medial amygdala in female rats

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ABSTRACT

Our previous study in male rats demonstrated that bilateral administration of flutamide, an androgen receptor (AR) antagonist, into the posterodorsal medial amygdala (MePD) increased the time sniffing male odors to as high as that sniffing estrous odors, eliminating the preference for estrous odors over male odors. This made us speculate that under blockade of AR in the MePD, testosterone-derived estrogen acting on the same brain region arouses interest in male odors which is otherwise suppressed by concomitant action of androgen. In cyclic female rats, endogenous androgen has been thought to be involved in inhibitory regulation of estrogen-activated sexual behavior. Thus, in the present study, we investigated the possibility that in female rats the arousal of interest in male odors is also normally regulated by both estrogen and androgen acting on the MePD, as predicted by our previous study in male rats. Implantation of either the estrogen receptor blocker tamoxifen (TX) or a non-aromatizable androgen 5α-dihydrotestosterone (DHT) into the MePD of ovariectomized, estrogen-primed female rats eliminated preference for male odors over estrous odors by significantly decreasing the time sniffing male odors to as low as that sniffing estrous odors. The subsequent odor discrimination tests confirmed that the DHT and TX administration did not impair the ability to discriminate between male and estrous odors. These results suggest that in estrous female rats estrogen action in the MePD plays critical roles in the expression of the preference for male odors while androgen action in the same brain region interferes with the estrogen action.

Introduction

Chemical communication plays an important role in the control of social and reproductive behaviors in many rodent species including rats. In particular, chemosensory cues trigger appetitive sexual behaviors, such as investigation and approach toward opposite-sex conspecifics. When given free access to soiled bedding or airborne odors from both sexes, sexually active male rats and estrous female rats spent more time sniffing those from opposite-sex conspecifics over those from same-sex conspecifics (Portillo and Paredes, 2004; Xiao et al., 2004). Chemosignals from members of the same species (pheromones) are detected by the vomeronasal organ (VNO) and the main olfactory epithelium (MOE). VNO neurons project to the accessory olfactory bulb (AOB) and MOE neurons project to the main olfactory bulb (MOB) (Scalia and Winans, 1975). Inputs from both the AOB and the MOB converge on the medial amygdala (MeA) (Pro-Sistiaga et al., 2007; Kang et al., 2009), and then neurons in the MeA project, in turn, to the medial preoptic area (mPOA) and the ventromedial hypothalamic nucleus (VMH) (Choi et al., 2005; Swanson, 2000), two brain areas which are prime candidates for regulation of appetitive and consummatory sexual behaviors (Harding and McGinnis, 2003, 2004; Xiao et al., 2005; Guarraci and Clark, 2006; Georgescu and Pfaus, 2006; Hull and Dominguez, 2007).

Lesion studies have suggested the importance of the MeA in the expression of female preference for male odors in mice (DiBenedictis et al., 2012), hamsters (Petrulis and Johnston, 1999) and rats (Kondo and Sakuma, 2005). For example, estrous female rats received the MeA lesion diminished the preference for odors from a sexually active male over a castrated male (Kondo and Sakuma, 2005), whereas male rats with MeA lesions maintained normal odor preference for estrous over anestrous females (Kondo and Sachs, 2002). Further evidence was provided by the studies in which preference for opposite-sex conspecific's odors was examined in relation to Fos expression induced by these odors in the brain regions along the above-mentioned olfactory projection pathways (Hosokawa and Chiba, 2005, 2007). When examined in sexually naïve rats of both sexes, opposite-sex odor induced-Fos expression was restricted to peripheral portions of the olfactory projection pathways (which include the MeA), while it extended to central portions, such as the mPOA, in sexually experienced

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subjects. On the other hand, male-directed odor preference of estrous females was observed irrespective of presence or absence of prior sexual experience, while female-directed odor preference of male rats was observed only in the subjects with prior sexual experience. These observations are not inconsistent with the notion that the MeA is a locus of neural substrates responsible for the expression of male-directed (female-type) odor preference, although the neural substrates responsible for the expression of female-directed (male-type) odor preference appear to exist somewhere else in the central portions of the olfactory projection pathways.

In both male and female rats, the preference for opposite-sex conspecific's odors was eliminated after gonadectomy and was restored by sex steroid replacement (Paredes et al., 1998; Xiao et al., 2004). These observations suggest that the expression of the odor preference requires not only chemosensory but also hormonal signals. Indeed, neurons containing androgen receptors (ARs) and estrogen receptors (ERs) are widely distributed at all areas of the olfactory projection pathways where pheromones are detected and processed (Shughrue et al., 1997; Simerly et al., 1990). Xiao et al. (2004) demonstrated that castrated male rats primed with estrogen and progesterone showed female-type odor preference. These data suggest that even male rats possess the neural substrates responsible for the expression of female-type odor preference without being defeminized during the perinatal "critical period" of sexual differentiation of the brain. The neural substrates, thus, may be activated to arouse interest in male odors when placed in female-like hormonal milieu.

Given that the MeA of the rats of both sexes is a locus of the neural substrates responsible for the expression of female-type odor preference, it is conceivable that in estrous female rats activation of ERs in this brain region by estrogens would be required to arouse interest in male odors. In male rats, on the other hand, Hosokawa and Chiba (2010) provided the idea that the arousal of interest in male odors induced via ER activation in the MeA by estrogenic metabolites of testosterone may normally be cancelled via concurrent activation of AR in the same brain region by testosterone and its androgenic metabolites. They demonstrated that in male rats bilateral administration of flutamide, an AR blocker, locally into the posterodorsal MeA (MePD), a sex steroid hormone receptor-rich subdivision of the MeA, eliminated preference for odors from an estrous female over those from a sexually active male. It is important to note that in this study the elimination of the male-type odor preference resulted from an increase in the time the males spent sniffing the male odors, not from a decrease in the time the males spent sniffing estrous odors. The male rats receiving flutamide, therefore, appear to have aroused interest in male odors as a result of the activation of solely ERs in the MePD, although existing interest in estrous odors which are likely regulated by brain regions other than the MeA remained unaffected.

It has been demonstrated that androgens are capable of antagonizing the actions of estrogens to influence behavioral responses in rodents. Administration of dihydrotestosterone (DHT), a non-aromatizable, androgenic metabolite of testosterone, to EB-treated, ovariectomized rats (Dohanich and Clemens, 1983; Kirkpatrick and Clark, 2011), mice (Luttge and Hall, 1976; Luttge et al., 1977), and hamsters (DeBold et al., 1978; Noble and Alsum, 1975) results in inhibition of estrogen-activated sexual behavior. There also are indications that changes in levels of endogenous androgens over the estrous cycle may normally serve to regulate the display of estrogen-activated sexual receptivity in intact female rats (Erskine, 1983). We thus hypothesized that in both male and female rats the MePD responds similarly to sex steroids and regulate the expression of female type odor preference; i.e., estrogen action in the MePD arouses interest in male odors while androgen action antagonizes the estrogen-activated arousal of the interest. The present study tested this hypothesis by investigating the effects of either the ER blocker tamoxifen citrate (TX) or DHT delivered locally into the MePD on the expression of sexual odor preference of estrogen-primed ovariectomized female rats.

Materials and methods

Animals

All of the male and female Long-Evans rats used in the present study were obtained from the institute for Animal Reproduction (Ibaraki, Japan) at 7 weeks of age, and housed in a controlled animal room at 24 °C under an artificial light–dark cycle of LD 12:12 (lights off at 07:00 h). Food and water were available ad libitum. Female rats used as experimental animals were initially kept in groups of 3 per cage and after brain surgery they were caged individually. Stimulus male and female rats were caged in single-sex groups of 2 to 3. Stimulus females were ovariectomized, and they were brought into estrous by injecting subcutaneously with 5 μg estradial benzonate (EB) and 500 μg progesterone (P) in 0.1 ml sesame oil 48 h and 4 h, respectively, prior to use. Stimulus males were left gonadally intact throughout the experiments. All experiments were conducted during middle of the dark portion of rats' photoperiod. All experimental procedures and animal housing were approved by the Institutional Animal Care and Use Committee of Sophia University and comply with the criteria established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996).

Brain surgery

Experimental rats were implanted stereotaxically with bilateral stainless steel 22-guide cannulae under ether anesthesia. Bilateral guide cannulae were inserted aiming the tip of the cannulae 2 mm above the MePD and the guide cannulae were attached to the skull surface using dental cement. Stereotaxic coordinates for the MePD were 4.6 mm anterior to interaural, 3.6 mm lateral to middle and 2.6 mm above interaural. The guide cannula of each side was plugged with an empty 28-gauge inner cannula protruding 2 mm beyond the end of the guide cannula until drug administration.

Odor preference test

Odor preference tests were carried out using an acrylic box (89(L) × 12(W) × 30(H) cm) consisted of 3 compartments (middle compartment and right and left side compartments). An experimental rat was placed in the middle compartment and stimulus rats (an estrous female and a sexually active male) were placed individually into the side compartments. Partitions between the compartments were composed of threefold opaque acrylic boards, and each of the boards had a round vent (3 cm in diameter) which allowed the passage of air. The vents on the three acrylic boards of each partition were arranged at alternate heights above the floor so that physical and visual contact between the experimental animal and the stimulus animals was completely prevented, although the experimental animal could smell and hear the stimulus animals. The air from each side compartment was introduced to the middle compartment through a cylinder (3 cm in inner diameter), which was fixed at the position of the vent (11.2 cm above the floor) of the partition board directly facing the middle compartment.

During a 5-min habituation period, a stream of air from the middle compartment to the side compartments was made using an electric fan fixed on the ceiling of the middle compartment. During a subsequent 5-min test period, the direction of the air stream was reversed; i.e., the air from the side compartments was introduced to the middle compartment. Preference for airborne odors was determined by measuring the times spent by the experimental rat poking their noses into the right and left cylinders. During the habituation period under air stream from the middle compartment to the side ones, experimental rats were never observed poking their noses into the cylinders, suggesting that when measuring preference by nose-poking time, the effects of sounds from stimulus rats, if any, are not significant. Before every odor