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Research report

The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression

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ABSTRACT

Social recognition manifests itself in decreased investigation of a previously encountered individual. Estrogen receptor alpha (ERα) knock out mice show deficient social recognition and anxiety. These data show that the ERα is involved in these effects, but they do not say anything about the brain sites important for these effects. In this study, an shRNA encoded within an AAV viral vector directed against the ERα receptor gene (or containing luciferase control), was injected bilaterally into the posterodorsal amygdala (MePDA) or the ventromedial nucleus of the hypothalamus (VMN) of female rats. An 81% reduction of ERα expression in the MePDA eliminated social recognition. Moreover, this diminution of ERα in the MePDA reduced anxiety in the light/dark choice test. In contrast, social recognition was unaffected after ERα knockdown in the VMN while aggressiveness against the juvenile was enhanced. In conclusion, social recognition and anxiety in female rats are modulated by the ERα in the amygdala. Moreover, aggression against juveniles but not against adults could, at least partly, depend on the ERα in the VMN.

1. Introduction

During the last decade the interest in the estrogenic regulation of behavioral processes beyond sexual behaviors has increased hugely. Estrogens have been shown to be implicated in cognitive functions [48], emotional reactions such as anxiety and fear [54] and social behaviors [80]. The role of estrogens in social recognition has also attracted some attention.

Social recognition is the ability to recognize familiar conspecifics and manifests itself in decreased investigation of a previously encountered individual [75]. The estrogenic regulation of social recognition in rodents has been investigated in a few studies with inconsistent results [30,47,64,74]. Nevertheless, a recent study showed that estradiol + progesterone improved social recognition in female rats [70]. A study in female mice, whose gene for the estrogen receptor alpha (ERα) or the estrogen receptor β (ERβ) had been knocked out, showed that they were deficient in social recognition compared to the wildtype [13]. Thus, there are data implicating ERs in social recognition, but these data gives no clue as to which of the ERs that is involved nor to the important brain site. Since there is ample evidence showing that the medial amygdala is important for social recognition [11,14,19], as well as being a structure where ERs are abundant [58], it is conceivable that estrogen actions within this structure are involved social recognition.

Anxiety is prominent among the emotions in which estrogens have been implicated. However, experimental results range from anxiogenic [29,35,53] to anxiolytic [35,46,59,78,82], and some studies report no effects at all [20,51,56]. The contradictory data could perhaps be explained by opposing actions of estradiol on the ERα and ERβ. Indeed, there are much recent data suggesting that stimulation of the ERβ consistently has anxiolytic effects [32,45,77]. It has also been proposed that estrogens are anxiolytic in safe environments, thereby facilitating sexual behaviors, and anxiogenic in threatening environments [54]. There is, then, no doubt that estrogens may exert important influences on anxiety-related behaviors. Whether the action is anxiolytic or anxiogenic may depend both on the environment and on the receptor subtype activated.

The medial amygdala is a crucial structure for emotional responses related to fear and anxiety [3,52,63]. Injection of estradiol into the medial amygdala has anxiolytic effects similar to those observed after systemic estradiol administration [23]. This observation suggests that the medial amygdala is an important target for the anxiolytic effects of estrogens. However the implication of ERα in this process remains to be demonstrated.

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The VMN may also be involved in the control of anxiety [26,49,67]. Whether estrogen receptors are involved in these actions is unknown, but since estrogens are known to modify the excitability of at least some neurons in the VMN [38,39,81] this is a definite possibility.

Another behavior modified by estrogens is aggression. In female rats as well as in mice it seems that estrogens increase aggression in some cases while estrogen + progesterone may reduce it [6,7,31,73]. Moreover, data from female ERα knockout mice show that aggressive behaviors are reduced under some circumstances and enhanced in others [57]. Neuroanatomical studies showed that the stimulation of the medial amygdala and the VMN increased aggression in rodents while lesions in these nuclei cause opposing effect [28,33,34,36]. Although both the medial amygdala and the VMN appear to modify aggressive behaviors, the role of estrogen receptors within these structures is unknown. Nevertheless, it can be proposed that estrogens within the VMN act to reduce aggressive behaviors. This would facilitate sexual interactions. Interestingly, both the display of lordosis and approach to a sexually active male depends on the ERα within the VMN [55,71], and both behaviors may require a reduced level of anxiety.

In the present series of experiments we determined how the absence of the ERα in the VMN or the MePDA affected social recognition, aggression and anxiety in ovariectomized female rats treated with estradiol + progesterone. To that end, a short hairpin RNA (shRNA) directed against the ERα gene coupled to a viral vector was infused into one of these structures. We decided to employ a sequential treatment with estrogen + progesterone in order to emulate the events occurring during the estrus cycle. Furthermore, estrogens induce the expression of progesterone receptors both in the medial amygdala [25,37] and the VMN, and this effect is basically mediated by the ERα [50]. Indeed, one of the most important cellular actions of estrogen is this induction of progesterone receptors. The omission of progesterone would, consequently, imply that one of the main actions of estrogens was ignored.

2. Materials and methods

2.1. Subjects

Male and female Wistar rats were obtained from Charles River WIGA (Sulzfeld, Germany). They were housed two per cage under a reversed light–dark cycle (12:12 h, lights off 1100). Food (RM1, Special Diets Services, Witham, Essex, England) and tap water were always available. Ambient temperature was maintained at 21 ± 1 °C and relative humidity was 55 ± 10%. Most females (250 g upon arrival to the animal facilities) were ovariectomized and some males (300 g upon arrival) were castrated under isoflurane anesthesia. The intact females were used for reproduction thereby assuring an adequate supply of juvenile rats for the social recognition test. Pups were weaned at 21 days and thereafter housed six per cage. For the behavioral tests, they were used between 23 and 26 days of age. All experimental procedures employed in the present experiment were approved by the National Animal Research Authority of Norway and were in agreement with the European Union council directive 86/609/EEC.

2.2. Stereotaxic surgery

About 2 weeks after ovariectomy some females received intracerebral infusions under ketamine/xylazine anesthesia (100 mg/kg and 10 mg/kg, respectively). After the females were fixed in a stereotaxic frame and small holes were drilled, a total of 2 μl of either an shRNA encoded within an AAV viral vector directed against the ERα gene (ERα shRNA) or with an shRNA containing a sequence direted against luciferase gene (AAV control) as well as an independent enhanced green fluorescent protein (EGFP) expression cassette to mark the injection site was infused. Bilateral cannulae (30 gauge) were aimed at either the VMN (coordinates were: anteroposterior, −2.80; mediolateral, ±.6; dorsoventral −9.6) or the mediodorsal posterior amygdala (MePDA; coordinates were: anteroposterior, −3.14; mediolateral ±3.6; dorsoventral, −8.2). Coordinates were based on the ([60]) atlas with the skull level. The infusion lasted 10 min and the cannulae were slowly withdrawn 5 min after the end of infusion.

2.3. Design of the shRNA employed for ERα silencing

An adeno-associated virus (AAV) vector was made to express a small hairpin RNA (shRNA) against either the sequence specific for the ERα gene (5′-GATCCCCGGCATGGAGCATCTCTACA CTTCCTGTCA TGTAGAGATGCTCCAT GCCTTTTTTGGAAT-3′ and 5′-CTAGATTCCAAAAAA GGCATGGAGCATCTCTACA TGACAGGAAG TGTAGAGATGCTCCATGCCGGG-3′) or the sequence specific for luciferase as control (5′-GATCCCCCCGCTGGAGAGCAACTGCAT CTTCCTGTCA ATGCAGTTGCTCTCCAGCGGTTTTTGGAA-3′ and 5′-CTAGTT-CCAAAAACCGCTGGAGAGCAACTGCAT TGACAGGAAG ATGCAGTTGCTCTCCA GCGGGGG-3′). The nucleotides specific for ERα or luciferase are underlined. The shRNA became incorporated into the neurons adjacent to the injection sites, and as soon as the shRNA was expressed, it blocked ERα gene expression for the rest of the animals' life. The shRNAs employed here have been described in detail elsewhere [55].

2.4. Design of experiment

Four groups of 13 rats each were used. (1) ERα shRNA infused into the VMN. (2) AAV control infused into the VMN. (3) ERα shRNA infused into the MePDA. (4) AAV control infused into the MePDA.

2.5. Behavioral testing

Three to four weeks after intracerebral infusion, experimental females were given estradiol benzoate (18 μg/kg) about 52 h before the tests were begun. Progesterone (1 mg/rat) was injected 48 h after estradiol and 4 h before the beginning of tests. Both steroids were purchased from Sigma, St. Louis, MO, dissolved in peanut oil and injected sc in 1 ml/kg and .2 ml/rat, respectively. These doses are known to produce a lordosis quotient close to 100 [2,69]. An equal number of experimental and control females were used simultaneously in each test. The VMN and MePDA groups were tested on different days.

2.5.1. Social recognition test

The females were housed in individual cages 1 h before the test. Shortly before the experimental session they were transferred to another room. A juvenile (23–26 days old) rat was put into a cylindrical plastic container (9 cm diameter, 11 cm high with open top) with air holes in the lid. Fifteen seconds before each exposure the lid was removed, and the container was then introduced into a female's cage in a horizontal position and left for 5 min. The juvenile invariably left the container, and all interactions with the experimental subject occurred consequently outside of the container. Fifteen min after removal the same juvenile was reintroduced into the female's cage in a clean container for another 5 min observation period. This was repeated until the female had been exposed to the juvenile 4 times. At the 5th exposure, a new juvenile was introduced and left for 5 min. The juvenile was placed into a clean container 5 min before being introduced into the female's cage. The same cylinder was used for all exposures, but it was thoroughly washed with unscented soap and then dried with paper towels between each exposure. Thus, there was no accumulation of odors in the container during the experiment. An incandescent light bulb provided a white light with an intensity of about 5 lux at the bottom of the cage. The females were left undisturbed in the room during the test without any observer present, and their behavior was videotaped for subsequent analysis.

The following behaviors were recorded: juvenile investigation (active sniffing and grooming of different parts of the juvenile), juvenile odor investigation (active sniffing of the container), container investigation (sniffing the outside of the container), sniffing other objects anywhere in the cage (exploration of the test cage), rearing, aggressive behaviors (the sum of kicking and biting the juvenile) and other active (locomotion, self-grooming, digging in bedding) as well as nonactive behaviors (sitting, lying down, both coded under the term of immobility) and freezing. The frequency and duration of these behaviors were determined from the videotapes with the help of the Observer XT software (Nodlus, Wageningen, the Netherlands).

2.5.2. Light/dark choice test

The test lasted 600 s and was performed in a two-compartment box consisting of one light chamber (20 cm × 30 cm × 45 cm), which was illuminated from the top with a white light producing an intensity of 600 lux at floor level, and one dark chamber (20 cm × 30 cm × 45 cm) with a light intensity of 5 lux at floor level. The chambers were separated by a black partition with a small opening (6 cm × 6 cm) at the bottom. Immediately after the test for social recognition, the animals were brought to an adjacent testing room and left undisturbed in their cage for 30 min. They were then introduced into the center of the dark chamber of the light/dark test apparatus, facing the light chamber.

A videotrack system (Ethovision Pro, Noldus, Wageningen, the Netherlands) determined the time the experimental subject spent in each chamber, the number of visits to each chamber, the latency to enter the dark chamber, and the latency to exit from the dark chamber (time after the first entry into the dark chamber until the first exit). The distance moved during the test, the mean velocity while moving, and the time of immobility were determined for both dark and light chambers and data are presented as total for the entire box.