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Brief communication

Modulation of male mouse sociosexual and anxiety-like behaviors by vasopressin receptors

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ABSTRACT

Although the involvement of two types of vasopressin (AVP) receptors, v1a and v1b, in neural regulation of social behavior is well documented in rodents, there is no report on combined actions of them in regulation of social behavior. In this study, we investigated behavioral differences between wild-type (WT) and v1a and v1b double knockout (dKO) mice. For this, we measured olfactory preference, sexual behavior with receptive females (four weekly tests) in an enriched large observation cage, and anxiety-like behaviors. No difference between WT and dKO mice was found in olfactory preferences for estrous female odor to male odor. Over all four mating tests, the number of mounts and pursuits after receptive females was significantly greater in dKO mice than in WT mice. In the elevated plus maze and the open field test, dKO mice showed lower anxiety-like behavior than WT mice. Finally, we measured approach behavior to several types of objects, figurines, and caged anestrous or estrous females placed in the open field apparatus. The only difference observed was that dKO mice spent longer in the vicinity of estrous females than did WT mice. These findings suggest that vasopressin receptors are involved in the regulation of sociosexual behavior, presumably partly mediated by emotional responses, in male mice.

1. Introduction

Brain nonapeptides vasopressin (AVP) and oxytocin (OT) have been well studied as neuromodulators that regulate social behavior in rats and mice. Although these peptides are very similar in structure, with seven identical amino acids out of nine, their functional roles in the regulation of social behavior are substantially different [1], caused by the difference in the distribution of their receptors in the central nervous system [2–4].

The effects of AVP application are more complicated because AVP has two types of receptors in the brain, v1a and v1b, and has cross-reactivity with OT receptors. Thus, many studies in the last decade have aimed to clarify the functions of v1a and v1b receptors in the regulation of social behavior. Deficiency of the v1a gene in mice has been associated with an impaired social recognition [5–7] and decreased anxiety-like behaviors in the open field test and elevated plus maze [7]. The administration of a v1a antagonist also decreases ultrasonic vocalization during mother-infant separation in rat pups [8]. It was reported that v1a plays a critical role in pair-bonding of monogamous voles, while overexpression of the v1a gene facilitates social recognition and affiliation behavior in rats [9] and mice [10]. However, only one report has demonstrated a distinctive impairment of social behavior in v1a-knockout (KO) mice, which reported a decreased interaction of v1a-KO mice when confronted with novel conspecifics than WT mice [11]. Similarly to v1a, deficiency of v1b decreases anxiety-like behaviors [12]. Furthermore, v1b-KO mice exhibit fewer aggressive behaviors than WT mice [12–14]. Since v1b is also expressed in adrenocorticotropic hormone-secreting cells, these phenomena may, at least partly, be mediated by dysfunction of the hypothalamus-pituitary-adrenal (HPA) axis [15].

Thus, the regulatory functions of v1a and v1b receptors are remarkably confined to certain aspects of social behavior. However, v1a and v1b receptors have both common functions and severally distinctive functions, so in the animal brain possessing both v1a and v1b, there may be synergistic functions different from those of v1a and v1b alone. In this study, to examine the combined effects of loss of these receptors, we compared sexual behavior, olfactory partner preference, and anxiety-like behavior in the open field test and elevated plus maze in mice deficient for both v1a and v1b genes (double knockout, dKO mice) with those in WT mice. Since no influence on sexual behavior was

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reported in v1b-KO mice [16], we used an enriched cage for sexual behavior tests, which promotes social interactions and prosocial behaviors. Furthermore, we examined the access behavior to objects or caged receptive females placed in an open field arena in dKO and WT mice.

2. Materials and methods

2.1. Animals

dKO mice deficient for v1a and v1b, which were produced by hybrid breeding of $v1a^{-/-}$ [17] and $v1b^{-/-}$ [18] mice and backcrossing to C57BL/6Jjcl, were kept in our laboratory (Fig. S1) [19]. WT mice of the same strain (C57BL/6Jjcl) for backcrossing were obtained from CLEA Japan, Inc. (Tokyo, Japan). The seven dKO and eight WT male mice used were 8–9 weeks old at the start of the experiment. All mice were housed under a controlled room temperature (23 °C ± 2) and in a reversed 12:12 light/dark cycle (lights on at 11:00 p.m.). Food and water were available ad libitum.

As stimulus male and female mice, 8-week-old C57BL/6 mice were also supplied from CLEA Japan, Inc., and kept in the same environment. After acclimation to our laboratory, all stimulus females were ovariectomized under isoflurane anesthesia. The females were subcutaneously injected with estradiol-17β benzoate (EB, 25 μg/0.05 ml of sesame oil) 48 h before and progesterone (P, 250 μg/0.05 ml of sesame oil) 3–5 h before each test. Stimulus males were used in sexually naïve intact. All experimentation and animal housing adhered to the guidelines for the Care and Use of Laboratory Animals of Teikyo University of Sciences, and were approved by our Institutional Committee for Experimental Animal Ethics.

2.2. Olfactory preference test

Olfactory preference tests and mating tests were weekly carried out 4 times on the same day, because behaviors measured in those tests were highly dependent on sexual experience. In every weekly trial, each subject was tested with different individuals as stimulus males and females.

The olfactory preference of the mice was assessed using the alternate choice paradigm in a three-chamber apparatus made of an acrylic box (25 × 25 × 15 cm), which consisted of a large compartment for subjects and two smaller chambers for stimulus mice. Partitions between the large and small compartments were doubled opaque plates that prevented visual and physical contacts between subject and stimulus mice, but with holes that enabled airborne odors to flow into the subject compartment. A fan was connected to the front wall of the apparatus through a corrugated tube to regulate airflow in the apparatus. A short transparent pipe (2 × 2 cm) was attached to the air inlet of the subject compartment to make more easily detect the subject's investigation of the stimulus odor. Before each test, the apparatus was cleaned with 70% ethanol.

Olfactory preference tests preceded mating tests on the same day. For each test, one stimulus male (intact) and one stimulus female (ovariectomized and primed with EB and P) was placed in a separate stimulus chamber, and one experimental male was placed in the subject chamber under dim red illumination. After 5 min acclimation, the preference test was started by turning on airflow. The time experimental mice spent nose-poking into the air-inlet pipes of each stimulus mouse was recorded by an event recorder for 5 min.

2.3. Mating test

Mating tests were conducted in an enriched environment apparatus (91 × 46 × 30 cm); the floor was covered with wooden chips, and the apparatus was equipped with a running wheel, a test-tube stand (jungle gym), and several tubes (tunnels). Each experimental mouse was acclimatized for 30 min per day for 5 days to the apparatus.

Each experimental mouse was tested once a week for 4 weeks in a dimly red-illuminated room. In each test, the experimental male was placed in the enriched apparatus for 5 min acclimation. The stimulus receptive female (used in the olfactory preference test on the day) was introduced to start the observation. The tests were unattended by experimenters, and were instead recorded for 30 min by a video camera. Sexual behavior (time spent mounting, number of mounts, intromissions, and ejaculations), rejections (including turning their body, upright posture, and fighting), staying in the vicinity (within 5 cm of females) longer than 5 s, physical contact, and pursuit of the stimulus female were measured. Time spent visiting and number of visits to the equipped objects (wheel, jungle gym, and tunnels) were also measured.

2.4. Elevated plus maze test

An elevated plus maze with four 30 × 5 cm arms (two of the four arms had 15 cm-high walls) was located 80 cm above the floor in the ordinary illuminated room. The test was started once the experimental mouse had been placed at the crossing. The activities (time spent in each arm) of experimental mice were recorded for 3 min by an overhead video camera.

2.5. Open field test

A circular open field (60 cm in diameter) surrounded by a 40 cm-high wall was used. Eight radiations were drawn on the white floor with a 30 cm concentric circle. The activities of experimental mice were recorded by an overhead video camera in the ordinary illuminated room. Ambulation (distance and speed) inside and outside the concentric circle was measured by tracking software (TopScan, Clever Sys Inc., VA, USA).

Experimental mice were subjected to 3 min open field tests under four conditions in order, as follows: 1) standard testing; 2) testing in the arena with two objects, a souvenir owl figurine (6 cm diameter × 6 cm height) placed 10 cm away from the wall, and a cactus (4 × 7 cm) placed 20 cm away from the wall; 3) testing in the arena with a stainless wire-mesh cage (10 cm diameter × 10 cm height) located at the center containing estrous (EB and P primed) females; and 4) tested in the arena with the stainless wire-mesh cage containing anestrous (ovariectomized) females. Each test was carried out on a different day. During the tests, ambulation distance and spent time inside and outside the center circle were recorded. For conditions two to four, the time spent in the vicinity of objects and nose contact were also recorded.

2.6. Statistics

Data from the olfactory preference test were analyzed using a three-way ANOVA (two genotypes × two stimuli × four repeated tests, the last two were repeated measures), and data from the mating test were analyzed using a two-way ANOVA (two genotypes × four repeated tests, the latter was repeated measure). In the case of a significant interaction, these tests were followed by multiple comparison post hoc tests (Tukey's tests). Data from the elevated plus maze and the open field test were analyzed using student's *t*-tests. Means presented here are followed by ± SEM. Latencies were analyzed by nonparametric tests and shown by medians.

3. Results

3.1. Olfactory preference test

The results (nose-poking time to intact male and receptive female mice) of olfactory preference tests are shown in Fig. 1. The ANOVA revealed a significant main effect of stimulus mice (nose-poking for estrous females was greater than that of intact males, $F_{1,13} = 90.95$,