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EFFECTS OF VASOPRESSIN ON FEMALE SEXUAL BEHAVIOR IN MALE RATSP. SÖDERSTEN^{1,*}, G.J. BOER², G.J. De VRIES², R.M. BUIJS² and P. MELIN³¹*Department of Psychiatry, Karolinska Institutet, S-141 86 Huddinge (Sweden),* ²*Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam (The Netherlands) and* ³*Ferring AB, S-200 60 Malmö (Sweden)*

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Intracerebroventricular (i.c.v.) injection of an Arg-vasopressin (AVP) antagonist did not stimulate female sexual behavior in adult castrated male rats treated with ovarian hormone but stimulated this behavior in male rats which were castrated on the day of birth. It is suggested that neonatal androgen stimulation in the male rat offsets the influence of AVP on female sexual behavior in the adult.

Arg-vasopressin (AVP) is produced in several brain regions outside the classical hypothalamic neurosecretory systems [5] and electron microscopical [2], autoradiographical [3] and electrophysiological [6] evidence suggest a neurotransmitter role for AVP in some of these brain areas. The AVP neurotransmitter system in the rat brain is sexually dimorphic [4]. The lateral septum (LS), for example, receives a much denser AVP innervation in the male than in the female [4] and lesions in the LS facilitate the display of female sexual behavior in female rats [8]. Since male rats show less female sexual behavior than females in response to ovarian hormones [10] and since AVP inhibits [12] and an AVP antagonist facilitates [11] female sexual behavior, it seems possible that the sexually dimorphic AVP neurotransmitter system may be functionally related to the sex difference in female sexual behavior. Consequently, in the present study attempts were made to facilitate female sexual behavior in male rats using an AVP antagonist.

Male Wistar rats were maintained with continuous access to food and water in an air-conditioned colony room in which the lights were off between 12.00 and 24.00 h. Thirty-five male rats (230–250 g) were castrated and 17–19 days later implanted with stainless-steel guide cannulae in the right lateral brain ventricle (as described in ref. 12). The rats were randomly divided into 5 groups (n = 7) and 2 groups were injected with 2 µg estradiol benzoate (EB, Sigma; dissolved in 0.1 ml sesame oil) and 3 groups with 10 µg EB s.c. at 15.00 h 2 days after the i.c.v. cannulation. Forty-two hours after the EB injection all rats received an s.c. injection of 0.5 mg progesterone

*Author for correspondence.

(P, Sigma; dissolved in 0.1 ml oil). Six hours after the P injection a group of 2 μ g EB-treated rats received an i.c.v. injection of 10 ng of the AVP antagonist deaminoethoxytocin (AAB, Ferring AB; dissolved in 2 μ l 0.9% NaCl [7] and the other group received an i.c.v. injection of NaCl. The 10 μ g EB-treated rats received i.c.v. injections of NaCl, 10 or 100 ng AAB. The rats were tested for female sexual behavior 20 min after the AVP injection by being placed with a sexually active male rat in circular (50 cm diameter) cages. The animals were observed until the stimulated male had mounted 10 times and a lordosis quotient: number of lordosis responses (concave back flexion, lateral tail deviation and neck extension) $\times$ 10, was calculated. In addition, proceptive behaviors [1], i.e. darting movements and earwiggling, were scored as present or absent. Tests were completed within 5 min.

Twenty male rats were castrated within 24 h of birth and 20 rats were sham castrated (abdominal incision). The sham castrated rats were castrated and the castrated rats were sham castrated (scrotal incision) at 70 days of age and 14 neonatally castrated and 14 control rats received i.c.v. guide cannulae at 90 days of age. Two days after the i.c.v. cannulation all rats were injected twice with 1 μ g estradiol (Sigma, dissolved in 0.1 ml oil and injected s.c.) at 20.00 and at 12.00 h the next day and with 0.5 mg P at 22.00 h. The rats were tested for lordosis behavior and proceptivity 6 h after the P injection at 04.00 h, i.e. in the middle of the L-phase of the light:darkness (LD) cycle. Seven randomly selected neonatally and 7 adult castrated rats were injected i.c.v. with 10 ng AAB. Seven neonatally and 7 adult castrated rats received i.c.v. NaCl injections. All rats were tested for lordosis behavior 20, 60 and 120 min after the AAB injection.

AAB had no effect on lordosis behavior in adult castrated rats. The mean $\pm$ S.E.M. lordosis quotients were: 30.5 ± 12.3 (2 μ g EB+NaCl), 48.0 ± 22.2 (2 μ g EB+10 ng AAB), 55.0 ± 13.5 (10 μ g EB+NaCl), 68.6 ± 13.4 (10 μ g EB+10 ng AAB) and 72.9 ± 12.8 (10 μ g+100 ng AAB). None of the rats showed proceptive behavior.

Fig. 1 shows that AAB facilitated the display of lordosis and proceptivity 20 min and lordosis 60 min after the injection in the neonatally, but not adult, castrated rats.

Parts of the vasopressinergic neuronal system is sexually dimorphic in adult rats and organized by testosterone (T) during development in a manner that is strikingly similar to that by which T organizes sexual behavior in the rat [4, 5]. Thus, parts of the adult rat brain, e.g. the LS, receive a much denser AVP innervation in the male than in the female [4, 5]. Lesions in the LS facilitate female sexual behavior in rats [8] as do i.c.v. injections of an AVP antagonist [11]. However, in this study we failed to facilitate female sexual behavior in adult castrated males with an AVP antagonist by using procedures which we have previously found to be effective in the female [11]. In addition, in unpublished studies no effect of the AVP antagonist was found in intact untreated or EB-treated males. These results do not support the idea that the sexually dimorphic AVP system in the rat brain is the anatomical substrate for the sex difference in female sexual behavior in rats.

If ovariectomized female rats are injected twice with 1 μ g estradiol followed by P and tested for sexual behavior the response is maximal during the D- and minimal during the L-phase of the LD cycle (P. Södersten et al., unpublished data). In this

Fig. 1. Mean $\pm$ S.E.M. lordosis quotients (number of lordosis responses to 10 mounts by males $\times$ 10) and number of animals showing proceptive behavior (earwiggling and darting) in groups of 7 neonatally and 7 adult castrated male rats. The rats were injected twice with 1 μ g estradiol (32 and 16 h) and 0.5 mg P (6 h) before testing and they were tested before (0 min) and 20, 60 and 120 min after an i.c.v. injection of 10 ng of the AVP antagonist AAB or NaCl. *Significantly different from adult castrated rats; P at least <0.05 , Mann-Whitney U -test (top) or Fisher exact probability test after χ^2 -test (bottom).

study we found that i.c.v. injection of an AVP antagonist facilitated the display of female sexual behavior in neonatally castrated male rats treated with two injections of estradiol and tested for sexual behavior during the L-phase of the LD cycle. The behavioral response to the AVP antagonist is maximal during this phase of the daily lighting cycle and related to the concentration of AVP in the suprachiasmatic nuclei of the hypothalamus (SCN), i.e. the neural generator of circadian rhythms [11]. In adult castrated rats, however, injections of the AVP antagonist had no behavioral effect and in unpublished studies we have been unable to affect any aspect of either male or female sexual behavior by injecting AVP or AVP antagonists during various phases of the LD cycle. It appears, therefore, that AVP influences sexual behavior in adult rats only if the animals have been castrated neonatally. Similarly, neonatal castration permits the expression of SCN-dependent rhythms in several reproductive neuroendocrine functions in the adult [10]. It has been suggested that neonatal androgen stimulation in the rat uncouples the SCN rhythm generator from the neural structures of sexual behavior in the adult [9] and we have argued the SCN may generate rhythmicity in sexual functions via its vasopressinergic projections in the brain

[10–12]. The present finding that adult castrated rats are refractory to the behavioral effects of AVP may be another example of the uncoupling of the rhythm generator through neonatal androgenic stimulation. However, the mechanisms whereby early stimulation by sex steroids might offset adult behavioral AVP sensitivity are unknown.

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