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A DAILY RHYTHM IN BEHAVIORAL VASOPRESSIN SENSITIVITY AND BRAIN VASOPRESSIN CONCENTRATIONS

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The concentration of Arg-vasopressin (AVP) in the suprachiasmatic nuclei of the hypothalamus (SCN), the neural generator of circadian rhythms, showed a daily rhythm, which was inversely related to the rhythm in lordosis, an aspect of sexual behavior shown by ovariectomized estradiol-17 β -treated female rats. A threshold dose of an AVP antagonist facilitated sexual behavior most effectively if injected intracerebroventricularly when the endogenous levels of AVP in the SCN were maximal and a threshold dose of AVP inhibited the behavior most effectively if injected when these levels were minimal. The results support the suggestion that AVP may be the neuropeptide whereby the SCN generate some behavioral rhythms.

The suprachiasmatic nuclei of the hypothalamus (SCN) generate circadian rhythms in behavior [14]. A variety of neuropeptides, all potential neurotransmitters, are produced by the SCN [4] and one of these, Arg-vasopressin (AVP) [6], shows a daily rhythm in the cerebrospinal fluid (CSF) [15], which is inversely related to the rhythm in sexual behavior shown by ovariectomized estradiol-17 β (E₂)-treated female rats [8]. In this study the concentration of AVP in the SCN has been measured throughout the light–dark (LD) cycle and correlated with behavioral sensitivity to an AVP antagonist and AVP.

Female Wistar rats (200–220 g) were ovariectomized and 1 week later implanted subcutaneously (s.c.) with E₂-filled implants and 4 days later tested individually for sexual behavior, i.e. lordosis (concave back flexion, lateral tail deviation and neck extension) every 3 h with male rats as described in [8]. Groups of ovariectomized rats were killed by decapitation at 4 h intervals and trunk blood was collected, centrifuged and the serum was stored at –20°C until assayed for AVP by radioimmunoassay [7]. The SCN were rapidly dissected out, put in 0.1 N HCl, sonified and stored at –20°C until assayed for AVP.

Ovariectomized rats were injected intracerebroventricularly (i.c.v.) with the AVP antagonist deaminoethyloxytocin (AAB, Ferring AB) [12] in the middle of the L

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phase and tested for lordosis in response to cervical stimulation [5, 11]. A glass rod, 4 mm in diameter with a round end was inserted into the vagina and briefly pressed against the uterine cervix while the rat was firmly held in the flank-perineum region [11]. The force needed for eliciting the response can be easily measured [5]. The maximum force applied was 6.5 N. This procedure provides a very strong stimulus and elicits the response even in the absence of ovarian hormones. Lordosis in response to cervical stimulation varies with endocrine state exactly as in response to male mounting. Also, the procedure offers an advantage over male mounting in that the stimulus intensity can be quantified [5, 11]. The rats were tested at 10 min intervals after the injection. Injection of 10 ng AAB i.c.v. had a pronounced effect in the 20 min test. This dose of AAB and time interval after the injection were, therefore, used for the following control experiments. One group of 6 rats received an i.p. injection of 10 ng AAB (dissolved in 0.5 ml 0.9% NaCl) and another group of 10 rats was injected with 10 ng oxytocin (Ferring AB, dissolved in 2 μ l 0.9% NaCl) i.c.v. and the rats were tested with cervical stimulation 20 min after the injections. Rats injected with AAB or oxytocin were given no treatment with ovarian hormones. Another group of 10 rats was injected with 0.8 μ g E₂ benzoate (EB, Sigma, dissolved in 0.1 ml sesame oil) s.c. 48 h before and 0.5 mg progesterone (Sigma, dissolved in 0.1 ml sesame oil) s.c. 6 h before being tested with male rats [8] and immediately after testing the rats received an i.c.v. injection of 10 ng AAB and they were retested with males 20 min later.

Two groups of ovariectomized rats were injected with 10 ng AAB i.c.v. either in the middle of the L or D phase and two other groups of rats were injected i.c.v. with a threshold dose of AVP (Ferring AB, 1 ng), selected on the basis of previous work [17], either in the middle of the L or D phase. The rats were tested at 10-min intervals after the injection of AAB or AVP. Animals treated with AVP were injected s.c. with 10 μ g EB and 0.5 mg progesterone (48 and 6 h before testing, respectively) to induce a high level of lordosis from which the inhibitory effect of AVP could be studied.

Fig. 1 shows that there was an inverse relationship between the display of lordosis and the concentration of AVP in the SCN. Serum concentrations of AVP were < 1.5 pg/ml.

Fig. 1. Mean $\pm$ S.E.M. lordosis quotients (number of lordosis responses at 10 months by males $\times$ 10) (●) shown by 7 ovariectomized estradiol-treated female rats tested at 3 h intervals and mean $\pm$ S.E.M. concentration of Arg-vasopressin (AVP) in the suprachiasmatic nuclei of the hypothalamus (SCN) (○) in groups of 4 ovariectomized rats decapitated at 4 h intervals throughout the light-dark (LD) cycle. *, Significantly different from lowest value; P at least < 0.05, Mann-Whitney U-test.

Thirteen out of 25 ovariectomized rats (52%) showed lordosis in response to cervical stimulation when tested during the L phase but significantly more, 22 out of 24 (91.7%, $P < 0.02$, χ^2 test), of a group of rats tested during the D phase displayed the response. There was, however, no difference in the force needed to elicit the response among responding animals tested during the L and D phase (6.3 ± 0.1 and 6.1 ± 0.1 N, respectively). Non-responding animals were assigned a value of 6.5 N in all subsequent experiments.

Fig. 2A shows that i.c.v. injections of AAB facilitated lordosis in a dose-dependent manner. Peripheral injection of AAB or i.c.v. injection of oxytocin had no effect. Animals pretreated with EB and progesterone showed a mean $\pm$ S.E.M. lordosis quotient of 23.3 ± 8.1 before and 51.1 ± 13.3 after an i.c.v. injection of AAB ($P < 0.05$, Wilcoxon test).

Fig. 2B, C shows that a threshold dose of AAB facilitated lordosis if injected during the L but not during the D phase and that a threshold dose of AVP inhibited lordosis if injected during the D but not during the L phase of the LD cycle.

A daily rhythm in the concentration of AVP in the SCN was demonstrated in this study and this rhythm was inversely related to the rhythm in sexual behavior shown by ovariectomized E_2 -treated rats [8]. Thus, peak levels of AVP were found during the L phase of the LD cycle when sexual receptivity reaches its minimum [8]. It is during this phase of the LD cycle that the SCN are maximally active metabolically [16] and electrophysiologically [9] and that the rhythm in the concentration of AVP in the CSF reaches its maximum [15]. Lesions of the SCN eliminate the rhythm in

Fig. 2. Induction of lordosis behavior in ovariectomized rats by application of force on the uterine cervix. A: effect of i.c.v. injection of 0 (Δ), 1 ($\blacktriangle$), 10 ($\circ$) or 1000 ($\bullet$) ng of the Arg-vasopressin (AVP) antagonist deaminoethyloxycytosine (AAB). B: effect of i.c.v. injection of 10 ng AAB and C, 1 ng AVP during the light ($\circ$) or dark ($\bullet$) phase of the LD cycle. Animals treated with AVP were pretreated with 10 μ g estradiol benzoate and 0.5 mg progesterone. *, Significantly different from NaCl-injected controls or B, animals injected during the dark or C, light phase; P at least < 0.05 , Mann-Whitney U-test after analysis of variance. There were 6–10 rats per group.

AVP levels in the CSF [1] and in sexual behavior [8]. Female rats with SCN lesion show high levels of sexual behavior throughout the LD cycle [8] and i.c.v. injections of AVP inhibit sexual behavior in receptive rats by acting on the brain, independently of its peripheral effects [17].

Here we found that i.c.v., but not peripheral, injections of an AVP antagonist, facilitated sexual receptivity in ovariectomized rats in response to artificial cervical stimulation in the absence of ovarian hormones as well as in response to mounting by male rats after treatment with ovarian hormones. This effect of the AVP antagonist was not replicated by i.c.v. injections of oxytocin. The effect of the AVP antagonist was dependent upon the phase of the LD cycle and maximal during the L phase when the endogenous levels of AVP in the SCN are maximal. Similarly, we found that the inhibitory effect of an i.c.v. injection of a threshold dose of AVP on sexual receptivity was dependent upon the phase of the LD cycle and maximal during the D phase when the endogenous levels of AVP in the SCN are minimal.

Thus, a behavioral rhythm, which is controlled by the SCN has been found to be inversely related to the rhythm in the endogenous levels of AVP in the SCN and behavioral sensitivity to an AVP antagonist and to AVP has been found to depend upon the phase of the rhythm in SCN AVP levels. These results support the suggested role of AVP in the generation of some behavioral rhythms by the SCN [17].

In unpublished studies we have found rhythms in the concentration of AVP in other brain areas some of which are directly innervated by the AVP fibers originating in the SCN, i.e. the organum vasculosum of the lamina terminalis, while others are not, i.e. the lateral septum [6]. Electronmicroscopical [3], autoradiographical [2, 18] and electrophysiological [10] evidence suggest a neurotransmitter role of AVP in these brain regions and sexual behavior is markedly facilitated by lesions in one of these regions, the lateral septum [13]. It is conceivable that AVP might act on one or several of these regions in the rhythmic control of lordosis.

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