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Intracerebral Implantation of the Anti-Estrogen CN-69, 725-27: Effects on Female Sexual Behavior in Rats[1]

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LUTTGE, W G *Intracerebral implantation of the anti-estrogen CN-69,725-27 Effects on female sexual behavior in rats* PHARMAC BIOCHEM. BEHAV 4(6) 685–688, 1976. – Ovariectomized adult female rats were stereotaxically implanted with double walled cannulas (22 ga outer and 27 ga inner) and tested for sexual receptivity (lordosis behavior) following SC estradiol benzoate and progesterone priming Implantation of the anti-estrogen CN-69,725-27 (c 6.5 μg) immediately after the first of 3 daily estrogen injections produced a dramatic inhibition of sexual receptivity when these implants were placed in the preoptic and anterior hypothalamic areas CN-69,725-27 implants had no effect on receptivity when implanted in the middle hypothalamus, mesencephalon or frontal cortex All inhibitory effects of the anti-estrogen were reversible within one day after removal of the CN-69,725-27 cannula from the brain

Ovarectomy CN-69,725-27 Estrogen Lordosis Sexual receptivity Progesterone Estradiol benzoate Anti-estrogen compound Anterior hypothalamus Preoptic area

SEXUAL receptivity in ovariectomized rats can readily be induced with exogenous estrogen and progesterone. The site of action for these steroids in the brain has been studied using a variety of techniques including brain lesions (e.g., [7, 13, 18, 20]), stereotaxic hormone implants (e.g., [5–8, 16, 19]) and radiolabeled hormone localization (e.g., [9, 15, 21]). Each of these techniques has its advantages and disadvantages. With brain lesions the behavioral effects that one observes may be due to interruption of fibers of passage, rather than the specific loss of hormone-responsive neurons in the lesion site. These lesions will also necessarily destroy neural tissue which may have little to do with hormone action and/or sexual behavior. Stereotaxic hormone implants usually destroy less neural tissue than lesions and the implanted steroid probably has little effect on the fibers of passage within the implant region. While these are certainly positive points, Davidson [5] has pointed out that with intracerebral estrogen implants, sexual receptivity can be induced with implants virtually anywhere within the brain. Furthermore, even those implants which had little influence on behavior can produce a significant stimulation of vaginal cornification, suggesting considerable leakage of the intracerebrally-implanted steroid into the general circulation. Intracerebral hormone implants can only be used to determine the sufficient sites of hormone action, they cannot be used to determine the hormone-responsive brain sites necessary for the induction of sexual receptivity. Radiolabeled hormone localization studies can provide rather elegant and detailed maps of the possible regional, subcellular and molecular sites of hormone action within the brain, but they do not identify the necessary sites of action. These studies also do not differentiate those hormone-concentrating neurons which are involved in reproductive behavior from all the other hormone-responsive neurons which may have little to do with behavior (e.g., those neurons involved in the feedback control of gonadotropin secretion).

In the present study we have approached the problem of localizing the intracerebral sites of action of estrogen in sexual behavior by capitalizing on the extremely potent actions of a new anti-estrogen, CN-69,725-27. This anti-estrogen has been shown to be a very effective inhibitor of ^{3}H-estradiol localization in neural and peripheral target tissues [9]. We reasoned that by stereotaxically implanting this anti-estrogen in suspected estrogen target regions within the brain we may be able to differentiate those regions which are required for estrogen induction of sexual receptivity from those regions wherein the actions of estrogen are sufficient, but not necessarily required for receptivity. This technique has been used with some success in studies attempting to localize the intracerebral sites for the positive feedback actions of estradiol on gonadotropin

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secretion [2,3]. Bloch and Davidson [4] also used this technique in an unsuccessful attempt to block male-typical sexual behavior with the anti-androgen cyproterone acetate.

METHODS

Animals

Adult CD female rats were purchased from Charles Rivers Laboratories (Wilmington, MA). All animals were housed individually with food and water available at all times The animal colony lights were left on from 2100 to 0900 hr. Based on this time clock all morning injections were scheduled between 0800 and 1000 hr and behavioral tests between 1300 and 1530 hr. When afternoon injections were scheduled they were given shortly after the completion of the behavioral testing.

Surgery

All females were bilaterally ovariectomized under ether anesthesia, and 1–2 weeks later they were anesthetized with sodium pentobarbital (45 mg/kg) and unilaterally implanted with 22 ga stainless steel guide cannulas. The 5 mm long guide cannulas were stereotaxically positioned so that they just entered the cortex, and they were rigidly held in place with wire, skull screws and dental cement. These cannulas were plugged with short, empty, L-shaped 27 ga cannulas The anti-estrogen was delivered to target regions in longer L-shaped 27 ga cannulas in which a 50.50 mixture of powdered cholesterol and CN-69,725-27 was packed by tapping the cannulas 20 times in small, steel, steroid-containing dishes. This mixture was selected on the basis of preliminary tests in which we found that 100% CN-69,725-27 implants seemed to block sexual receptivity everywhere that it was implanted, including cerebral cortex, while 10% CN-69,725-27 implants failed to inhibit receptivity. The steroid-containing cannulas could be exchanged for the short plug cannulas while the rat was unanesthetized and, after experience with handling and pretest implantations, with apparently little stress to the animal. Weighing these cannulas on a Cahn G-2 electrobalance revealed that the implanted material averaged 13.0 ± 0 3 μg. Thus, the amount of CN-69,725-27 implanted into the brain should have been approximately 6.5 μg.

Procedure

Beginning at least one week after stereotaxic implantation all females were given a series of weekly tests for sexual receptivity. Each test consisted of placing the female into a 30 × 30 × 60–90 cm clear Plexiglas testing arena containing a sexually vigorous stud male. Males were permitted to mount the females 10 times and the lordosis quotient (L/M × 100) was computed for each female. Only mounts with pelvic thrusting were counted. If the male failed to either initiate or continue mounting, the female was moved to another testing arena containing a different male. During these pretests the threshold dose for estradiol benzoate (E_2 B) therapy was established for each female. This dose was defined as the minimum dose which, when given SC for 3 days, would not stimulate receptivity (i.e., L/M $\leq$ 20) unless an SC injection of 500 μg progesterone was given 4 hr before the test. In many of these pretests the rats were given experience with the implantation procedure either by exchanging the plug cannula with another plug or with a cholesterol-containing cannula immediately after the first E_2 B injection each week. These cannulas were removed after the behavioral test. All SC injected steroids were dissolved in oil (0.05–0.1 cc).

After the E_2 B thresholds were established (Range. 0.125–1 μg/day × 3 days), hormone injections were continued at weekly intervals. Prior to the first E_2 B injection each week the female either received an implant of the 50 50 cholesterol·CN-69,725-27 mixture or pure cholesterol. Twenty-four hr after the last E_2 B injection all females received SC injections of 500 μg progesterone followed 4 hr later by a standard 10 mount test for sexual receptivity (CN-Test). Immediately after testing the steroid-containing cannulas were removed and the females injected with a threshold dose of E_2 B. On the next day all females were again injected with progesterone and tested 4 hr later for sexual receptivity (Post-Test). If the female failed to display a L/M score of at least 50 during the post-test, then the results from that week's testing were not included in the final analysis. In many cases the results from a given implant test were replicated in the same animal to verify the findings. In these cases the average results were used in the final data analysis. At the conclusion of the experiment all females were perfused with 10% Formalin, the brains sectioned at 50–75 μm and the sections stained with thionine to aid in the confirmation of the implantation sites

RESULTS

The results from the behavioral tests are summarized in Table 1 and the location of the tips of the steroid-containing cannulas are illustrated in Fig. 1 (the frontal cortex implant sites are not illustrated). Anti-estrogen implants in the preoptic area and anterior hypothalamus produced a dramatic inhibition of sexual receptivity, while similar implants in middle hypothalamus, various mesencephalic sites and frontal cortex had no effect on receptivity. High lordosis quotients were observed in all groups during the post-tests, indicating that the anti-estrogen had no permanent effects on receptivity. Four females with either preoptic or anterior hypothalamic implants which had previously displayed an inhibition in receptivity following CN-69,725-27 implantation were retested with cholesterol implants The average L/M ($\bar{X}$ ± SEM) displayed during these tests was 95 ± 2.9, a score which is clearly equivalent to that displayed during the earlier post-tests. Thus the anti-estrogen inhibition of receptivity was not due to the non-specific actions of an implant in the brain.

DISCUSSION

The present demonstration that anti-estrogen implantation in the preoptic and anterior hypothalamic regions inhibit sexual receptivity suggests that these brain areas contain estrogen receptors whose activation is required for the induction of receptivity. Recent work in this laboratory has shown that pretreatment with CN-69,725-27 leads to a nearly complete inhibition of ^{3}H-estradiol uptake into nuclear fractions isolated from preoptic and anterior hypothalamic samples [9]. Systemic treatment with a variety of anti-estrogens has also been shown to effectively inhibit both estrogen- and androgen-induced sexual receptivity [1, 10, 11, 22, 23]. Our demonstration of a possible required action of estrogen in the preoptic and

TABLE 1

LORDOSIS QUOTIENTS (L/M X 100, $\bar{X} \pm$ S E M) FOR OVARIECTOMIZED E2B PRIMED FEMALE RATS DURING TESTS WITH (CN TEST) AND 24 HR AFTER REMOVAL (POST-TEST) OF INTRACEREBRAL ANTI-ESTROGEN IMPLANTS

Site	n	CN Test	Post-Test	CN vs Post
Preoptic Area & Ant Hypothalamus	13	20 8 ± 4 0	90 8 ± 3 5	$p<0$ 001
Mid Hypothalamus	11	67 3 ± 1 2	77 3 ± 5 7	$p>0$ 05
Mesencephalon	11	77 2 ± 5 7	75 4 ± 4.7	$p>0$ 05
Frontal Cortex	15	86 0 ± 6 5	92 7 ± 2 7	$p>0$ 05

anterior hypothalamic areas is consistent with the early estrogen intracerebral implantation studies. However, as discussed in the Introduction these findings have recently been challenged by Davidson [5], who noted that estrogen implants even in the cerebral cortex could lead to a significant facilitation of receptivity. Furthermore, all of these intracerebral estrogen implants usually produced at least some uterine stimulation, suggesting estrogen leakage from the brain into the peripheral circulation.

Lesions in the preoptic and septal areas have been shown to facilitate the induction of sexual receptivity [13,18], while electrical stimulation of these regions inhibit receptivity [12, 14, 25]. Intracerebral implants of the DNA-dependent RNA synthesis inhibitor, actinomycin-D, into the preoptic area at 12, but not at 21 hr after SC injection of E_2B has also been shown to effectively block the induction of receptivity [24]. Thus, combining these findings with our present results using intracerebral implants of the anti-estrogen CN-69,725-27 strongly suggests that the preoptic, septal and anterior hypothalamic regions of the brain are involved in the necessary actions of estrogen in the induction of sexual receptivity.

At the present time one can only speculate as to the possible mechanism(s) of estrogen action in this system. For example, estrogen could enter the target cells in the preoptic, septal and anterior hypothalamic regions and bind first with cytosolic and then nuclear receptors. We have shown that CN-69,725-27 can effectively block this interaction [9]. The estrogen-nuclear receptor complex could then stimulate the production of specific m-RNA sequences which in turn could stimulate the production of specific proteins. Actinomycin-D treatment should effectively block this action of estrogen. The newly produced estrogen-specific proteins could then facilitate the induction of sexual receptivity by somehow suppressing the tonic inhibitory actions of the preoptic, septal and anterior hypothalamic regions Lesions in these regions could facilitate receptivity [13,18] by removing this tonic in-

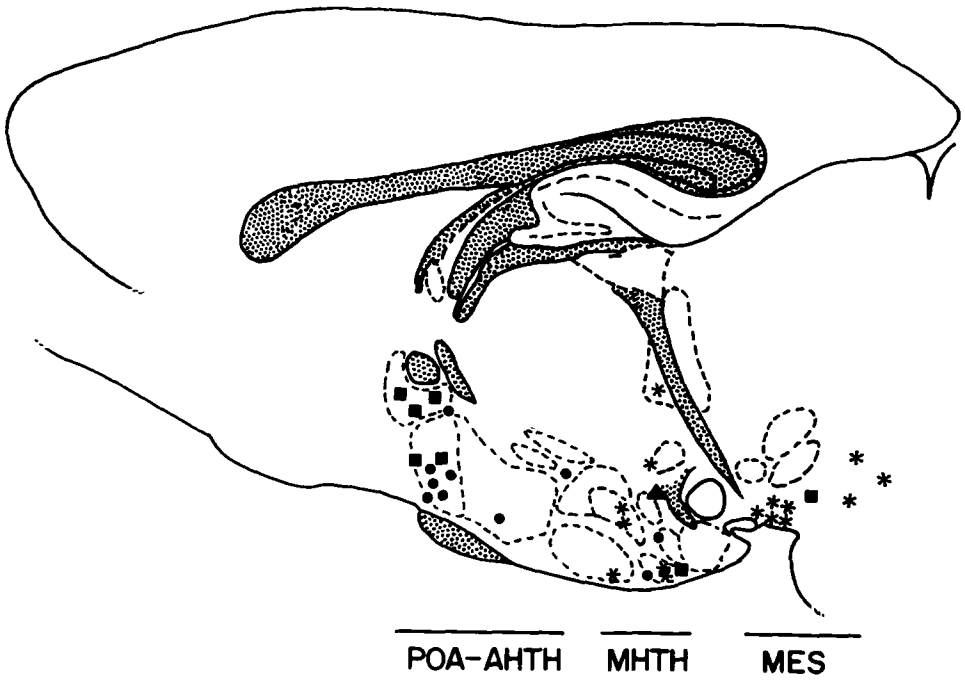

FIG. 1 Sagittal view (6) of rat brain illustrating the location of the cannula tips in the preoptic-anterior hypothalamic (POA-AHTH), middle hypothalamic (MHTH) and various mesencephalic (MES) sites. Implants in the frontal cortex are not illustrated. Symbols indicate the receptivity score (L/M X 100) during the CN-Test asterisks (70–100), triangles (50–60), squares (30–40) and circles (0–20).

hibition, while electrical stimulation in these regions could suppress receptivity [12, 14, 25] by potentiating the tonic inhibitory actions. Although this theory is clearly consistent with the data, its verification of refutation must await further experimentation.

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