Induction of Ovulation by Electrochemical Stimulation in Androgenized and Spontaneously Constant-Estrous Rats[1] (34245)

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Several studies have revealed that the failure of ovulation in androgen-sterilized rats, rats with anterior hypothalamic lesions, and aging or continuously illuminated rats with constantly cornified vaginas and micropolycystic ovaries, is attributable to an alteration in the neural regulation of hypophyseal activity (1–8). Both the pituitary gland and ovaries of anovulatory female rats with constant vaginal cornification function normally upon transplantation into normal rats (5, 9–11).

Although vaginal cornification and failure of ovulation are symptoms common to the rat sterilized with neonatal androgen treatment and the spontaneously constant-estrous rat, several differences have been observed. Barraclough and Gorski found that electrical stimulation of the basal arcuate ventromedial nuclear complex in androgen (high dosage) sterilized rats would induce ovulation only after preliminary progesterone treatment (6). In rats sterilized with a low dosage of androgen, electrical stimulation of the basal hypothalamus induced ovulation without priming with progesterone, whereas stimulation of the preoptic area failed to induce ovulation (7). Rats in "constant estrus" induced by continuous illumination also ovulated after electrical and electrochemical stimulation of amygdala (12). Rats sterilized with a low dose of androgen (10 μg, fifth day) ovulate in response to copulation (13). However, ovulation is not induced in this group by treatment with progesterone (6, 7, 14), which is effective in the spontaneously constant-estrous rat (15). Because of these differences it was of interest to compare the thresholds and responsiveness of these two groups and of cyclic rats (16) to stimulation applied directly to the brain.

Materials and Methods. Forty-one mature androgen-sterilized rats (3–4 months of age) and 12 spontaneously constant-estrous mature Sprague-Dawley rats were used. The animals weighed 280–400 g and were maintained in a light-controlled room. Vaginal smears were taken every morning for 2 months, and the continued presence of cornified cells was confirmed. Testosterone propionate (50 μg) in oil was used for sterilization, administered when the rats were 5 days of age. Progesterone was injected subcutaneously at a dosage of 1.25–2.5 mg in sesame oil as described in the "Results."

For electrical and electrochemical stimulation of the rat brain, concentric bipolar stainless steel electrodes were used. The basic method of stimulation was described by Terasawa and Sawyer (16). Electrical current was supplied by a Grass S-4 stimulator. The parameters were the following: direct current (dc) or 200 cps monophasic rectangular pulses of different amplitudes, pulse durations of 0.5 msec applied for 15-sec on and off over 30 min. Current was monitored routinely with a cathode ray oscilloscope.

Pentobarbital (31.5 mg/kg of body wt) was injected intravenously 15 min before 14:00. The animals were fixed in the stereotaxic apparatus when asleep. Stimulation was applied unilaterally through electrodes in-

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serted into the hypothalamus and preoptic region, using the atlas of Albe-Fessard, *et al.* (17). Ovulation was determined by direct observation of the presence of ova in the oviducts the next morning. Brains were fixed in 10% formalin, sectioned, and stained to check the electrode sites.

Results. 1. Effect of electrochemical stimulation on ovulation in the androgen-sterilized rat. Electrochemical stimulation of the medial preoptic area in androgen-sterilized rats was highly effective in causing ovulation. None of the 12 rats stimulated with 10–100 μA dc failed to ovulate (Table I). The number of ova observed in both oviducts averaged 5.1 with a range of 2–9. No correlation was observed between the current applied and the number of ova. The threshold current inducing ovulation by medial preoptic stimulation was 10 μA, which was lower than that in control rats. Ovulation was also induced by electrochemical stimulation of the arcuate nucleus and median eminence in androgen-sterilized rats. Here, the threshold of direct current for ovulation was the same in both control and androgenized rats (100 μA). The mean number of 5 ova in the latter was less than that in control rats.

2. Effect of electrical stimulation on ovulation in the androgen-sterilized rat. Electrical stimulation of the medial preoptic area and the arcuate nucleus–median eminence region also caused ovulation in a limited number of androgenized rats, although its effectiveness was less than that of electrochemical stimulation. The thresholds for ovulation (400 μA) in both areas were considerably higher than in control animals (Table II).

3. Electrochemical stimulation in spontaneously constant-estrous rats. In spontaneously constant-estrous rats electrochemical stimulation of the medial preoptic region and arcuate nucleus–median eminence area again induced ovulation (Table I), but the dc threshold in the preoptic area was 100 μA, considerably higher than that of either controls or androgen-sterilized rats. The arcuate nucleus threshold was not ascertained, but one rat ovulated at 100 μA. The mean number of ova in this small group (10.5) was quite similar to the control.

TABLE I. Comparison of Thresholds of Ovulation and Number of Eggs Ovulated in Three Groups of Rats by Electrochemical Stimulation of the Brain.

Stimulation site	Electrochemical stimulus dc, 60 sec (μA)	Control rats[a]			Androgen-sterilized constant-estrous rats			Spontaneously constant-estrous rats		
		Total no.	No. ovulating	No. ova/ovulating rat (mean)	Total no.	No. ovulating	No. ova/ovulating rat (mean)	Total no.	No. ovulating	No. ova/ovulating rat (mean)
Medial preoptic region	5				3	0	—			
	10	6	0	—	2	2	7.5			
	20	6	5	12.2	3	3	4.7	1	0	—
	30	6	5	11.2	4	4	4.8	1	0	—
	50	2	2	11.0	2	2	5.5	2	0	—
	100				1	1	6.0	1	1	11.0
Arcuate nucleus and median eminence	20	1	0	—						
	30	1	0	—						
	50	2	0	—	1	0	—			
	100	4	3	11.0	3	2	5.0	1	1	10.0

[a] Control data from Terasawa and Sawyer (16) but the constant-estrous experiments were performed concurrently.

TABLE II. Thresholds of Electrical Stimulation of Ovulation in Control and Androgen-Sterilized Rats.

Stimulation site	Electrical stimulus 0.5 msec, 200 cps; 15 sec on/off, 30 min (μA)	Control rats[a]			Androgen-sterilized constant-estrous rats		
		Total no.	No. ovulating	No. ova/ovulating rat (mean)	Total no.	No. ovulating	No. ova/ovulating rat (mean)
Medial preoptic region	50	4	0	—			
	100	5	4	10.5			
	200	5	4	6.2			
	300	5	3	9.7	2	0	—
	400	6	6	9.1	3	1	6.0
Arcuate nucleus and median eminence	50	1	0	—			
	100	4	0	—			
	200	5	0	—	1	0	—
	300	5	5	8.5	2	0	—
	400	5	5	10.0	2	1	8.0

[a] Control data from Terasawa and Sawyer (16) but the androgen-sterilization experiments were performed concurrently.

4. Effects of progesterone on ovulation in androgen-sterilized and spontaneously constant-estrous rats. In contrast to their relatively lowered sensitivity to electrochemical stimulation, the androgen-sterilized rats were resistant to progesterone, a hormonal stimulus which induced ovulation in the spontaneously constant-estrous rat (Table III). None of the androgenized rats ovulated even when the progesterone dosage was raised to twice the level effective in the spontaneously constant-estrous group.

Discussion. Although rats in constant estrus induced by continuous illumination have the ability to ovulate upon electrical or electrochemical stimulation as demonstrated by Bunn and Everett (8) and Velasco and Taleisnik (12), androgen sterilized rats have been reported to ovulate upon electrical stimulation of the preoptic area only when progesterone is given (14, 16). However, in the present results, 100% of rats sterilized with a low dosage of androgen ovulated upon electrochemical stimulation. They appeared to remain in a preovulatory phase, with pituitaries needing only a triggering activation from the central nervous system for ovulation to occur. Although progesterone treatment increases pituitary LH content in sterile rats (6, 7, 14) it was not necessary for electrochemical stimulation of ovulation in sterile rats. Furthermore, progesterone did not act as the key for triggering LH release in androgenized rats as it does in spontaneously constant-estrous animals.

How should the lack of spontaneous ovulation in androgenized rats be interpreted? They respond to exogenous gonadotropins with a reduced number of ova (5) and their pituitary glands contain a low level of LH

TABLE III. Failure of Progesterone to Induce Ovulation in Androgen-Sterilized as Compared with Spontaneously Constant-Estrous Rats.

Progesterone dosage (mg)	Androgen-sterilized constant-estrous rats			Spontaneously constant-estrous rat		
	Total no.	No. ovulating	No. ova/ovulating rat (mean)	Total no.	No. ovulating	No. ova/ovulating rat (mean)
1.25	6	0	—	3	3	8.7
2.5	6	0	—			

(14, 18). These factors might limit the number of ova following electrical or electrochemical stimulation, but pituitary–ovarian limitations do not prevent ovulation in response to copulation (13) or the application of electric current to the arcuate nucleus (16). From the present work it would appear that transmission from preoptic region to hypothalamus is facilitated, since the preoptic threshold is lowered. Therefore the absence of spontaneous ovulation in these rats must result from the failure of generation of the normal cyclic triggering stimulus in the brain, presumably in the preoptic region.

A second question is why progesterone does not induce ovulation in androgenized rats as it does in spontaneously constant-estrous rats. Androgenized rats have also been shown to be quite insensitive to progesterone's elevation of sexual receptivity (19), so their brains may be insensitive to progesterone in general. In normal cycling rats, components responsive to progesterone have been observed in the basal hypothalamus and medial preoptic area by multiple unit recording (Terasawa and Sawyer, unpublished). Responses in this system interpreted as a positive feedback mechanism are lost to the hypothalamus by "anterior deafferentation." Positive feedback influences of progesterone on ovulation [*i.e.*, advancement of the critical period (20)] are lost on anterior deafferentation which separates the hypothalamus from preoptic region but maintained following far anterior cuts which have the preoptic–hypothalamic connections intact (Van Rees, personal communication). The androgenized rat in some ways resembles a case of early anterior deafferentation of the hypothalamus. Since these rats have not experienced cyclic changes in vaginal smears, a progesterone feedback loop has never been established or activated. Spontaneously constant-estrous rats, which had established progesterone feedback loops as evidenced by normal ovarian cycles between puberty and the onset of persistent estrus, will ovulate in response to progesterone alone.

Whereas the androgenized rat is insensitive to progesterone but maintains a low threshold of electrochemical stimulation of the preoptic region, the spontaneously constant-estrous rat appears to be sensitive to but lacks progesterone and, in its absence, maintains an elevated threshold. When exogenous hormone is supplied the threshold drops to the level at which spontaneous ovulation occurs—an initial positive feedback mechanism in the biphasic action of progesterone (20–22). In the androgen-sterilized rat the inherent cyclicity of the female preoptic region is never attained where in the spontaneously constant-estrous rat it is established and lost, perhaps through some failure of progesterone secretion.

Summary. Female rats sterilized by neonatal treatment with small doses of androgen are resistant to the ovulatory action of progesterone, but they readily ovulate a small number of eggs in response to low-threshold electrochemical stimulation of the preoptic region. On the other hand, spontaneously constant-estrous rats have a high threshold to electrochemical stimulation which is lowered to the spontaneously ovulating level by treatment with progesterone.

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