Effects of Hypothalamic Stimulation, Hormones, and Drugs on Ovarian Function in Old Female Rats (34260)

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Aschheim (1) demonstrated that transplantation of ovaries from young into old rats did not result in reinitiation of estrous cycles, providing evidence that the loss of normal reproductive function in old rats did not originate at the ovarian level. Aschheim (2) also reported that ovulation and cycling activity could be restored in old constant estrous rats by LH administration. This suggested that the primary cause of ovarian failure in the old rats was due to deficient secretion of LH by the pituitary.

Since anterior pituitary function is controlled by the hypothalamus, changes within the hypothalamus may account for the loss of normal reproductive function in old female rats. This possibility was investigated by direct electrical stimulation of the preoptic area of the hypothalamus and by use of several drugs and hormones.

Materials and Methods. The animals used were 12–15 months old, multiparous Sprague-Dawley rats obtained from Spartan Animal Farms, Haslett, Michigan and from Holtzman Company, Madison, Wisconsin. The rats were housed in steel cages, fed a diet of Wayne Lab Blox pellets (Allied Mills, Chicago) and tap water *ad libitum* and were given oxytetracycline (Pfizer) in their drinking water (0.2 mg/ml) once weekly as a prophylactic measure against respiratory infections. When the rats reached 20 months of age, daily vaginal smears were taken and recorded. Rats found to be not cycling but in constant estrus for 1 month or more were selected for these experiments.

Progesterone and an antiandrogen (Cyproterone acetate, Schering A. G., Berlin)[5] were dissolved in sesame oil, while epinephrine was suspended in sesame oil. Reserpine and thyroxine were administered in aqueous solutions. Progesterone, 4.0 mg, was injected daily for 3 days, and the other substances were injected subcutaneously in daily doses for 10 days as follows: Cyproterone acetate, 10 mg; epinephrine, 0.25 mg; reserpine, 50 mg; thyroxine 1, 5, and 10 μg.

The preoptic hypothalamus was electrolytically stimulated by passing monophasic square wave pulses, 150 μ A intensity, 1.0 msec duration and 100/sec for 2 min through bipolar stainless steel electrodes. While stimulation was in progress, the current and waveform were continuously monitored on a Tektronics 564 oscilloscope. The bipolar electrodes were composed of wire 0.05 mm thick. All electrodes were permanently implanted into the brains of the rats 2 days before stimulation. At the time of electrode implantation a laparotomy was performed, and the ovaries were examined for corpora lutea. No corpora lutea were observed in any of the old constant estrous rats. One week after stimulation a laparotomy was performed again, and the ovaries were examined for corpora lutea as an indication that ovulation had occurred. When the animals were killed, the location of the electrodes was determined histologically.

Results. Treatment with progesterone, known to act centrally in facilitating ovulation, caused 6 of 10 rats to ovulate (Table

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TABLE I. Effects of Drugs, Hormones, Castration, and Stimulation of Preoptic Area of the Brain on Ovulation and Estrous Cycles in Old, Anovulatory, Constant Estrous Rats.

Treatment	No. of rats	No. showing ovulation	Observed ovarian function
Progesterone, 4 mg daily for 3 days	10	6	Few days diestrus followed by constant estrus
Epinephrine, 0.25 mg daily	22	11	Many normal cycles
Ovariectomy	3	0	Constant anestrus
Reserpine, 50 mg daily	6	0	Constant estrus
Cervical stimulation	6	0	Constant estrus
Cyproterone acetate, 10 mg daily	10	0	Constant estrus
Stimulation of preoptic hypothalamus	5	3	Few days diestrus followed by irregular pattern
Thyroxine, 1, 5, or 10 μg daily	18	0	Constant estrus

I). Epinephrine, also known to act centrally on many neuroendocrine functions, produced ovulation in 11 of 22 rats. Unlike progesterone injections, which resulted in only a few days of diestrus, epinephrine administration resulted in many normal appearing estrous cycles.

Treatments which exerted no observable effects were Cyproterone acetate administration, stimulation of the uterine cervix, reserpine and daily administration of different doses of thyroxine. Ovariectomy resulted in a permanent anestrous condition as determined by vaginal smears, demonstrating that estrogen secretion from the ovary was responsible for the constant estrus. In three of five 2-year-old females with atrophic ovaries and a history of 3 months of constant estrus, stimulation of the preoptic area of the hypothalamus caused ovulation. Implantation of electrodes without stimulation did not result in ovulation.

Discussion. Induction of ovulation in old rats by administration of progesterone and epinephrine and by electrical stimulation of one of the neural centers controlling ovulation suggests that changes in brain function, possibly in the preoptic area, are at least partially responsible for the decline of ovarian function. Everett and Quinn (3) showed that the preoptic area of the hypothalamus controls ovulation in the rat. Progesterone is believed to stimulate release of LH by a central action on the hypothalamus (4), and epinephrine has also been observed to stimulate ovulation in several species (5). We cannot rule out entirely the aging of the ovaries as a contributory factor to their own senescence. However, the observation that the ovaries of old rats can be reactivated indicates that the cause of ovarian failure lies at a higher level.

The inability of an antiandrogen, Cyproterone acetate, to counteract the constant estrus observed in these animals appears to eliminate the possibility that androgens are responsible for the constant estrus of old rats. The anestrous condition observed after ovariectomy suggests that the adrenals, which may be capable of secreting estrogens with advancing age (6) did not secrete sufficient estrogen to stimulate the vagina. In addition, decreased thyroid function does not appear to be the cause of ovarian failure, since administration of thyroxine at several doses had no effect on ovarian function. It appears therefore, that age changes in endocrine organs have little to do with the decline of ovarian function in old female rats.

It appears from this study that the absence of normal ovarian function and the resultant constant estrus in many old female rats is probably due to changes in neural activity in the brain. The nature of these neural changes are not entirely clear, and it is uncertain whether they are located in the hypothalamus or in areas impinging on the hypothalamus. It is possible that neural changes occur

in old rats which are the reverse of those that take place at puberty. Frolkis (7) observed a decrease in neural substances with aging, and this may result in reduced stimulation to LRF secretion by the hypothalamus. In suppport of the central role of the brain in this process, we have recently observed that in old constant estrous rats, hypothalamic content of LRF is very low whereas FSH-RF content is very high as compared with 3-month-old cycling rats during estrus (8).

Summary. Old female rats in constant estrus were given different treatments in an attempt to induce ovulation. Subcutaneous injections of progesterone for 3 days or epinephrine for 10 days, induced ovulation in 6 out of 10 and 11 out of 22 rats respectively, with normal appearing estrous cycles observed in many epinephrine-treated rats. Electrical stimulation of the preoptic area of the hypothalamus produced ovulation in 3 out of 5 old rats. Injections of reserpine, thyroxine or cyproterone acetate, and cervical stimulation, failed to induce ovulation, whereas ovariectomy resulted in anestrus. These observations suggest that changes in hypothalamic function account for constant estrus in old female rats.

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