NOTE

Failure of Intrahypothalamic Implants of Antiestrogen, MER-25, to Inhibit Androgen-Sterilization in Female Rats

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Synopsis

Two-day-old female rats were given bilateral implants of small pellets each containing 5 µg MER-25 in bone wax in the medial preoptic to ventromedial areas of the hypothalamus. At the same time, the rats received a single subcutaneous injection of 50 µg testosterone propionate. Ovaries of the rats were examined by laparotomy for the presence of corpora lutea at 50 days of age. At 100 days, ovaries were removed and studied histologically. Sterilizing action of testosterone propionate was not inhibited by intrahypothalamic implants of MER-25, at least the present experimental conditions.

Testosterone administration to neonatal female rats induces a syndrome of sterility, characterized by failure of ovulation and formation of corpora lutea. The animals so treated are acyclic when adult, showing a masculine pattern of gonadotropin secretion. Female rats treated with estrogen neonatally also develop a syndrome which is at least superficially indistinguishable from that produced by androgen (For reviews, see Takewaki, 1962; Gorski, 1971; Arai, 1973). Recent studies suggest that testosterone administered neonatally might exert its effects on the brain via conversion to estrogens. Demonstration of aromatizing enzymes in the hypothalamus and limbic system of fetuses and adult animals (Naftolin et al., 1971, 1972; Reddy et al., 1974; Weisz and Gibbs, 1974) and failure of non-aromatizable androgens to induce masculinization in neonatal female rats (McDonald and Doughty, 1972a) are in harmony with this concept. Findings reported by McDonald and Doughty (1972b, 1973) that the effects of testosterone subcutaneously injected to neonatal female rats were inhibited by an antiestrogen, MER-25, likewise administered subcutaneously, lend support to this hypothesis.

Since it is well established that the hypothalamus is the primary site of "masculinizing" action of androgen given neonatally (Wagner et al., 1966; Nadler, 1972; Hayashi and Gorski, 1974), experiments were conducted to determine whether the effects of testosterone propionate injected subcutaneously into newborn female rats can be inhibited by concurrent implantation of MER-25 into the hypothalamus.

Materials and Methods

Female rats of the Sprague-Dawley strain were given intrahypothalamic implants of MER-25 between 24 and 48 hrs after birth. Stereotaxic implantation was performed under cold anesthesia with the animals head stabilized in the head holder positioned in a stereotaxic apparatus for adult rats. The head holder was mask made of silicone rubber (Flexicon, The G-C Chemical Mfg. Co. Ltd., Tokyo, Japan). The outer wall of the mask was painted with dental resin (Repairsin, The G-C Chemical) to give an enough rigidity. A conical mold made of Modelling Compound (The G-C Chemical) or Flexicon was
glued to the tip of each ear bar to fix the head holder (Fig. 1A). The head holder was so adjusted as to keep the basal part of the hypothalamus horizontal and its mediasagittal plane vertical (Fig. 1B). Head holders of different sizes were needed for pups of different sizes. Bilateral implantation of pellets was accomplished by using 27-gauge tubings (0.4 mm in outer diameter and 0.2 mm in inner diameter) attached to electrode carrier of the stereotaxic apparatus. A mixture of MER-25, charcoal bone and bone wax (C. D. Lukens Co., St. Louis, USA) in ratio of 50%, 20% and 30% by weight, respectively, was melted by warming and then cooled between two slide glasses kept 0.25 mm apart by a ring of metal wire. Pellets were made just before implantation by pressing the tip of the 27-gauge tubing into the sheet of the mixture. The tubing bearing a pellet at the tip was inserted into the brain toward the desired site and the pellet was extruded immediately by means of a stylet. The retracted tubing was examined each time to insure that the pellet had been left behind in the brain.

Implantation was aimed to either the preoptic area or the ventromedial hypothalamus. Parameters used were 5.0 mm or 6.0 mm beneath the bregma, 0.8 mm right and left to midline, and 1.0 mm anterior to the bregma for the preoptic area, and 1.0 mm posterior to the bregma for the ventromedial hypothalamus. Preliminary trials were carried out in litter males prior to implantation in female pups. The males were sacrificed immediately after operation, the brains removed and examined under a dissecting microscope. Half of females of each litter were given intrahypothalamic pellets containing MER-25, while the other half received pellets containing 23% of charcoal bone but no MER-25. Immediately after implantation, The rats received a single subcutaneous injection of 50 μg TP dissolved in 0.02 ml sesame oil or 0.02 ml oil vehicle only.

The average weight (+S.E.) of 21 pellets was 9.8 ± 0.8 μg. Since each pellet contains 50% of MER-25 by weight and pellets were implanted bilaterally, each animal received about 10 μg of MER-25 on an average.

The pups were weaned around 28 days of age. From the day of vaginal opening on, daily vaginal smears were recorded. At 50 days of age, each animal was laparotomized to examine the status of the ovaries. When the rats became approximately 100 days old, the right ovary was removed from each animal and weighed. Exactly 3 weeks later, the remaining ovary was removed and the ovarian compensatory hypertrophy (OCH: difference in weight between the 2 ovaries/weight of the right ovary) was calculated. The ovaries were fixed in Bouin’s solution, imbedded in paraffin, sectioned at 8 μ and stained with Delafield’s hematoxylin and eosin. On the basis of histological structure of the ovaries, animals were classified into 3 groups: (1) animals with polyfollicular ovaries, (2) animals with a few corpora lutea (CL) in one side of the ovaries, although main ovarian components were follicles of varying sizes and interstitial tissue, and (3) animals having some CL, follicles of varying sizes and interstitial tissue in their ovaries. The animals of the first 2 groups were regarded as sterilized. Incidence of sterility (IS: number of sterilized rats/number of treated rats) was calculated for each group.

The animals were sacrificed at 8–10 months old. The brains were removed after perfusion with 0.9% saline followed by 10% formalin. Frozen sections cut at 60 μ were stained with carbol fuchsin. Localization of the implanted pellets was checked histologically.
The animals having pellets in asymmetrical positions or whose brain histology could not be examined were discarded. As illustrated in Figure 2, the intracerebral loci of pellets varied considerably among different individuals, being in the basal part of the diagonal band of Broca (DBB), medial preoptic area (POA), optic chiasma (CO), anterior hypothalamic area (AHA), ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), zona incerta (ZI) or the posterior part of lateral hypothalamic area (LHA).

Results

At 50 days of age, 7 out of 13 female rats which had received injection of 50 µg TP along with intrahypothalamic implants of MER-25 neonatally exhibited no CL in their ovaries. CL were also absent in 4 out of 6 50-day-old rats given 50 µg TP and bone wax implants within 2 days after birth. By contrast, those animals receiving oil injection neonatally invariably showed CL in their ovaries, regardless of whether they had received implants of bone wax (8 animals) or of MER-25 (13 animals) concurrently. At 100 days of age, ovaries of all the 6 animals given 50 µg TP along with bone wax, and of 12 out of 13 animals receiving 50 µg TP together with MER-25 were of the sterile type (Table 1). The remaining one rat of the latter group had large cystic follicles and some CL in both ovaries but the left ovary removed at 121 days of age was smaller in weight (33.1 mg) than that of the controls (62.5 ± 4.3 mg), indicating that gonadotropin secretion in this animal was permanently reduced.

In the rats treated with 50 µg TP neonatally both the right (21–23 mg) and the left ovaries (31–39 mg) were significantly smaller in weight than the respective ovaries of the oil-injected and untreated rats (31–40 mg and 53–63 mg, respectively). However, there was no significant difference in magnitude of the ovarian compensatory hypertrophy among these groups (Table 2).

Eight out of 19 females receiving neonatal injection of 50 µg TP had no vaginal opening until 100 days of age. In the remaining 11 rats, vaginal opening occurred at 38.8 ± 1.5 days of age, which was not significantly different from the age of vaginal opening in 21 rats given oil injection neonatally (40.6 ± 0.9 days).
Table 1. Incidence of sterility* (IS) after neonatal administration of testosterone propionate and MER-25

<table>
<thead>
<tr>
<th>Neonatal treatments</th>
<th>Intra-hypothalamic implants</th>
<th>State of ovaries and IS</th>
<th>at 50 days of age</th>
<th>at 100 days of age</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>CL present</td>
<td>CL absent</td>
<td>IS (%)</td>
</tr>
<tr>
<td>Oil</td>
<td>Bone wax</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oil</td>
<td>50% MER-25</td>
<td>13</td>
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</tr>
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<td>Bone wax</td>
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<td>4</td>
<td>67</td>
</tr>
<tr>
<td>50 µg TP</td>
<td>50% MER-25</td>
<td>6</td>
<td>7</td>
<td>54</td>
</tr>
</tbody>
</table>

* Rats having polyfollicular ovaries without CL or exhibiting polyfollicular ovaries still with a few CL were regarded as sterilized.
** No. of animals. CL: Corpora lutea.

Table 2. Ovarian weights and ovarian compensatory hypertrophy (mean ± S.E.) in neonatally androgenized and MER-25 implanted rats.

| Neonatal treatments | Intra-hypothalamic implants | Ovarian weight (mg) | Ovarian compensatory hypertrophy (%)
|---------------------|-----------------------------|---------------------|-----------------------------------|
|                     |                             | Right ovary | Left ovary | Right ovary
|                     |                             |            |            |            |
| None                | None                        | 8          | 40.0 ± 4.0a| 62.5 ± 4.3a| 60.5 ± 9.4 |
| Oil                 | Bone wax                    | 8          | 33.9 ± 2.2| 55.1 ± 2.5| 66.4 ± 13.0|
| Oil                 | 50% MER-25                  | 13         | 31.2 ± 1.9| 53.1 ± 2.6| 72.2 ± 6.2 |
| 50 µg TP            | Bone wax                    | 6          | 23.0 ± 2.7b| 38.5 ± 5.1b| 69.6 ± 13.1|
| 50 µg TP            | 50% MER-25                  | 13         | 21.2 ± 1.9c| 31.3 ± 1.7c| 56.4 ± 11.1|

The right ovary was removed exactly 3 weeks prior to removal of the left ovary. The difference in weight between a and b or c is statistically significant (a vs b, p < 0.01; a vs c, p < 0.001).

Discussion

The present findings are not in agreement with those reported by McDonald and Doughty (1972b, 1973) that the permanent effects of neonatal androgenization were inhibited by prior treatment with MER-25. In the hamster, Gottlieb et al., (1974) reported that the sterilizing effects of neonatal administration of androgen could not be antagonized by MER-25. Anyhow it is felt that the interpretation of androgen sterilization as an exclusively estrogen function must be accepted with some reservation.

However, at least a part of difference in the outcome between the experiment of McDonald and Doughty (1972b, 1973) and the present ones might be due to the differences in dosage of androgen and MER-25, and the route of administration of MER-25. According to McDonald and Doughty (1972b), subcutaneous injection of 100 µg MER-25 was not strong enough to suppress the effects of 30 µg TP injected simultaneously at 5 days of age. Accordingly, the 50 µg dose of TP injected subcutaneously at 2 days of age in this experiment might be too large to be suppressed by the intrahypothalamic implants containing 10 µg MER-25.

It is also possible that TP administered subcutaneously would exert its effect on diffused
areas of the hypothalamus (Hayashi and Gorski, 1974) or the brain, while intrahypothalamic implants of MER-25 would act only on limited areas around the pellets. Accordingly, implanted MER-25 might be less effective than that injected subcutaneously. The report that incidence of sterility following neonatal subcutaneous androgen administration was reduced by repetitive subcutaneous injections of pentobarbital but not by its intrahypothalamic implantation (Sutherland and Gorski, 1972) appears to lend support to this concept.

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References

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