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Failure of Intrahypothalamic Implants of an Estrogen Antagonist, Ethamoxytriphetol (MER-25), to Block Neonatal Androgen-Sterilization[1] (39403)

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It is well documented that androgen administration to neonatal female rats evokes sterility characterized by polyfollicular anovulatory ovaries and persistent vaginal cornification (neonatal androgenization; 1-4). Recently, several workers have postulated that the conversion of androgen to estrogen is a prerequisite for induction of neonatal androgenization (5-10). One of the reports supporting this postulation is that subcutaneous injection of an estrogen antagonist, ethamoxytriphetol (MER-25), 6 hr prior to testosterone propionate (TP), could block androgenization (8). However, Brown-Grant (11) reported that he obtained only a capricious influence of the estrogen antagonist on induction of neonatal androgenization. The present author has also failed to observe a blocking effect of MER-25 on neonatal androgenization (12). In the present series of the experiments, although the amount of MER-25 per pellet was increased and that of TP was reduced to threshold level for induction of anovulatory sterility, the ineffectiveness of MER-25 on neonatal androgenization was confirmed.

Materials and methods. Fifty-eight female rats of the Sprague-Dawley strain were divided into six groups at 4 days of age (day of birth = Day 1). Group I received a subcutaneous injection of 0.02 ml of sesame oil at 5 days of age (8 rats); group II received a subcutaneous injection of 30 μg of TP at 5 days of age (5 rats); group III received bilateral implantation of intrahypothalamic paraffin micropellets (sham operation) at 4 days of age and a subcutaneous oil injection (7 rats); group IV received the same operation as group III (sham) and a subcutaneous injection of 30 μg of TP at 5 days of age (12 rats); group V received intrahypothalamic micropellets of MER-25-paraffin mixture at 4 days of age and a subcutaneous oil injection at 5 days of age (11 rats); and group VI received the same implants as group V but followed by a subcutaneous injection of 30 μg of TP at 5 days of age (15 rats).

TABLE I. MEANS (±SE) OF DAY OF VAGINAL OPENING (VO) AND ONSET OF PERSISTENT VAGINAL ESTRUS (PVE) AND INCIDENCE OF STERILITY (IS) IN RATS RECEIVING INTRAHYPOTHALAMIC IMPLANTATION OF PARAFFIN MICROPELLETS WITH OR WITHOUT MER-25 AT 4 DAYS OF AGE AND A SUBCUTANEOUS INJECTION OF TESTOFTERONE PROPIONATE (TP) AT 5 DAYS OF AGE.

Group	Intrahypothalamic implants	Subcutaneous injection	Number of treated rats	S/F[a]	VO (day)	Onset of PVE (day)	IS[b] (%)
I	None	Oil	8	F(8)	41 ± 1	–	0
II	None	30 μg TP	5	S(5)	41 ± 2	74 ± 6	100
III	Sham	Oil	7	F(7)	40 ± 1	–	0
IV	Sham	30 μg TP	12	S(9)	38 ± 1	71 ± 4	75
				F(3)	38	–	
V	MER-25	Oil	11	F(11)	40 ± 1	–	0
VI	MER-25	30 μg TP	15	S(10)	31 ± 2**	59 ± 4*	67
				F(5)	37 ± 3	–	

[a] S = sterile and F = fertile rats. Number of animals are indicated in parentheses.

[b] $IS = \frac{\text{No. of sterile rats}}{\text{No. of treated rats}} \times 100\%$. *, ** Significantly earlier ($P < 0.05$, < 0.02, respectively) compared with group IV.

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The method of the intracranial implantation of paraffin pellets was a slight modification of the technique explained previously (12). The MER-25-paraffin mixture was tamped into stainless steel tubings (22 gauge, 0.4-mm i.d.) which contained a precut loading stylet. Weights of 11 control pellets were measured by an ultramicrobalance (Mettler ME 22) to the nearest 0.1 μg and the average weight (±SE) was 84.7 ± 1.8 μg. Since MER-25 was mixed with paraffin in 50% by weight, the amount of MER-25 per pellet averaged 42 μg. The animals were weaned at 22 days of age, and daily smears were recorded following the day of vaginal opening. To examine the ovarian compensatory hypertrophy (OCH), right ovaries were removed at 90 days of age, the remaining left ovaries 3 weeks later regardless of the day of sexual cycle. About 4 weeks after the last ovariectomy (around 120 days of age), the animals were sacrificed and the presence of intrahypothalamic micropellets was determined histologically. Ovaries were fixed in Bouin's solution, cut serially in paraffin, and stained with Delafield's hematoxylin and eosin. Those animals having polyfollicular ovaries without fresh corpora lutea (CL) were determined sterile. The significance of differences was tested with Student's *t* test.

Results. The injection of 30 μg of TP to 5-day-old female rats seemed a threshold amount of the steroid for induction of sterility, since only 9 out of 12 animals (75%) that received the sham-implantation at 4 days of age and a subcutaneous injection of TP at 5 days of age (group IV) became sterile by 90 days of age. On the other hand, TP injection of 5-day-old rats (group II) invariably induced sterility. Out of those 15 animals which were implanted with MER-25-paraffin mixture at 4 days of age and given a subcutaneous TP injection at 5 days of age (group VI) only 10 rats (67%) developed sterility; the remaining 5 had fresh CL in their ovaries (Table I). The micropellets of MER-25-paraffin mixture were found in suprachiasmatic nucleus (SC), anterior hypothalamic area (AHA), dorsomedial hypothalamic nucleus (DMH), or posterior hypothalamic nucleus (PH) of those rats which had received a TP injection neonatally and which had newly formed ovarian CL (Fig. 1A, shaded circles). Animals in which the micropellets were located in SC, paraventricular nucleus (PVH), DMH, or PH in those rats which were given a TP injection neonatally became sterile (Fig. 1A, open circles). In TP-injected rats which received paraffin pellets without MER-25, and which contained fresh ovarian CL at autopsy, the micropellets were located in DMH or PH. In rats which were sterile at autopsy the pellets were located in POA, AHA, VMH, DMH, or PH (Fig. 1B, shaded and open

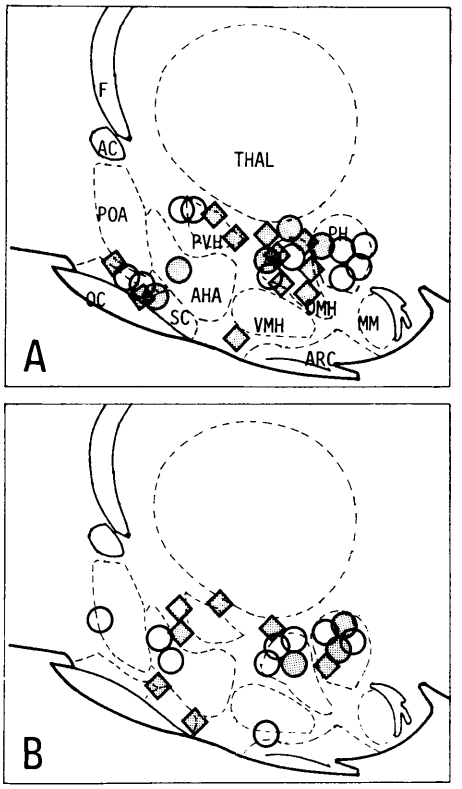

FIG. 1. Schematic parasagittal drawings of the hypothalamus showing loci of paraffin micropellets containing MER-25 + carbon marker (A) or marker only (B, sham operation). Those animals which had received a subcutaneous injection of 30 μg of TP at Day 5 became sterile (open circles) or fertile (shaded circles), while those which had been given an oil-vehicle injection became fertile invariably (shaded squares). Abbreviations: AC, anterior commissure; AHA, anterior hypothalamic area; ARC, arcuate nucleus; DMH, dorsomedial nucleus; F, fornix; MM, medial mammillary nucleus; OC, optic chiasm; POA, preoptic area; PH, posterior hypothalamus; PVH, paraventricular nucleus; SC, suprachiasmatic nucleus; THAL, thalamus; VMH, ventromedial nucleus.

TABLE II. MEANS (±SE) OF BODY WEIGHTS AT 90 DAYS OF AGE, OVARIAN WEIGHTS AND OVARIAN COMPENSATORY HYPERTROPHY (OCH) OF 3-WEEKS-INTERVAL IN THE SAME RATS AS IN TABLE I.

Group[a]	Number of rats	S/F[a]	Body weight (g)	Ovarian weight (mg)		OCH[b] (%)
				Right	Left	
I	8	F(8)	258 ± 10	38.7 ± 3.6	59.4 ± 4.3	56 ± 6
II	5	S(5)	233 ± 12	21.7 ± 2.1**	24.8 ± 1.7***	16 ± 5***
III	7	F(7)	236 ± 13	34.4 ± 2.3	54.0 ± 3.6	57 ± 6
IV	12	S(9)	260 ± 7	22.8 ± 1.8**	29.1 ± 1.5***	31 ± 7*
		F(3)	256	49.6	61.5	19
V	11	F(11)	255 ± 7	39.2 ± 2.6	60.9 ± 4.1	57 ± 6
VI	15	S(10)	253 ± 9	21.8 ± 2.0***	32.2 ± 3.6***	53 ± 13
		F(5)	262 ± 14	42.5 ± 7.5	43.6 ± 5.0	21 ± 29

[a] See Table I.

[b] $\text{OCH} = \frac{\text{Left ovarian weight} - \text{right ovarian weight}}{\text{Right ovarian weight}} \times 100\%$. *, **, and ***, Significantly smaller than respective controls (groups I, III, and V, $P < 0.02$, < 0.01, and < 0.001, respectively).

circles, respectively). No significant correlation seemed to be present between localization of intrahypothalamic micropellets and sterility.

The days of vaginal opening (VO) and onset of persistent vaginal estrus (PVE) of those rats which had received MER-25 and TP were significantly earlier than in those of sham-operated, TP-injected rats (Table I). Ovarian weights of the sterile rats from groups II, IV, and VI were also significantly smaller than those of respective controls which had received oil injection (groups I, III, and V, respectively). OCH of sterile rats of groups II and IV was significantly smaller than those of respective controls (groups I and III, respectively; Table II).

Discussion. As stated in the previous report, intrahypothalamic pellets with MER-25 (around 5 μg/pellet) implanted bilaterally in the 5-day-old females which immediately received subcutaneous injection of 50 μg of TP failed to block sterilizing effect of the androgen (12). The ineffectiveness of MER-25in the previous experiment (12) could be attributed to the fact that the amount of MER-25 was too small to block the overthreshold amount of TP (i.e., 50 μg) given immediately after operation. However, it is shown in the present experiment that a larger amount of MER-25 (42 μg/pellet on an average) also failed to block the sterilizing effect of a smaller amount of TP (30 μg) which was given 24 hr after the intrahypothalamic operation. The fact that 5 out of those 15 animals which had been given MER-25 and TP (group VI) had fresh CL in their ovaries as adults may be attributable not to the blocking influence of the antiestrogen on androgen-sterilization but to the threshold amount of TP applied to induce sterility, since 3 out of those 12 rats which had received sham-operation and TP-injection (group IV) also had fresh CL. It is concluded, therefore, that the interpretation of androgen sterilization as an exclusively estrogen function must be accepted with some reservation.

Summary. The influence of an estrogen antagonist, MER-25, on androgen sterilization was examined. Micropellets of MER-25-paraffin mixture, containing 42 μg of the antiestrogen per pellet on an average, were implanted bilaterally into the hypothalamus of 4-day-old female rats which received a subcutaneous injection of 30 μg of TP at 5 days of age. The amount of TP was, therefore, considered a threshold level for sterilization. In MER-25 implanted, TP-injected females, 10 out of 15 showed sterility, and the remaining 5 had fresh CL in their ovaries. No significant block by the antiestrogen of androgen-sterilization was detected. The previous interpretation of androgen-sterilization as an exclusively estrogen function must be, therefore, accepted with some reservation.

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