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Evidence of hypothalamic involvement in the mechanism of transplacental carinogenesis by diethylstilbestrol

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Summary

Disruption of hypothalamic sex differentiation in the fetus is one hypothesis to explain female reproductive system anomalies and cancer arising from prenatal exposure to diethylstilbestrol (DES). To further test this hypothesis, breeding performance and behavior were monitored in a colony of mice exposed prenatally to DES, using a schedule previously shown to produce anomalies and cancer of the female reproductive system. Fertility decreased with age more rapidly in DES-exposed females than in control females. DES-exposed females were less accepting of the male than control females. These observations support the hypothesis of abnormal hypothalamic sex differentiation as a basic mechanism in DES transplacental carcinogenesis.

Keywords: diethylstilbestrol; mice; prenatal; carcinogenesis; hypothalamus

Introduction

Several hypotheses have been proposed to explain the mechanism by which prenatal exposure to diethylstilbestrol (DES) produces cancer in the female reproductive system [11]. One hypothesis is that DES, a synthetic estrogen, causes abnormal sex differentiation of the hypothalamus in the fetus. The consequent imbalances in hormonal production might explain the developmental abnormalities and cancer of the female reproductive system [11], as well as the pituitary tumors reported for one of the animal models [13]. Two other effects of abnormal hypothalamic sex differentiation, namely, altered sex behavior and the delayed anovulatory syndrome described for rats [4], have not previously been reported in a murine model of DES-induced cancer. To explore these two characteristics in an animal model with anomalies and cancer comparable to those seen in women exposed prenatally to DES [8] the breeding characteristics of this model will be reported. The breeding colony of mice used in this study was established to conduct embryo transfer experiments for purposes explained elsewhere [11].

Materials and Methods

Strain CD-1 mice were maintained on a light cycle with a 10 h dark period from 16:00 h to 02:00 h. A male was placed in a cage with 4 females overnight and the females checked for a VP (vaginal plug) the next morning. At 17 days, 16 h after the assumed time of conception [6], DES (Sigma Chemical Co., St. Louis,

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Table I. Comparison of VP frequency in DES-exposed and VEH-exposed mice during 4-week periods starting at 4 to 7 weeks.

Age weeks	DES		DES by age[a]	VEH		VEH by age[a]	DES vs VEH[b]
	VP	No VP		VP	No VP		
4–7	99	801		44	260		$P > 0.1$
8–11	41	535	$P < 0.02$	71	457	$P > 0.5$	$P < 0.001$
12–15	0	204	$P < 0.001$	12	184	$P < 0.01$	$P < 0.001$
16–19	0	108		10	134	$P > 0.5$	$P < 0.01$

[a]Probability by χ^2 of only chance difference between this and preceding age group.
[b]Probability of only chance difference between DES and VEH groups of this age.

MO) was injected in a dose of 1 μg/g body weight. The DES solution was prepared by dissolving 5 mg of DES powder in 5 ml of olive oil with 0.1 ml of absolute alcohol to enhance solubility. An equal volume of the vehicle was injected into pregnant mice that would serve as controls. The offspring of these pregnancies were raised to mating age (Tables I and II) to provide mice exposed prenatally to DES ('DES-exposed mice'), or vehicle ('VEH-exposed mice'). These prenatally exposed female mice were mated to control mice, intact or vasectomized, overnight and checked the next morning for the presence of a VP. The standard mating system was to place the male overnight in a cage containing 4 females. Alternatively, a single female in estrus, selected by vaginal smear, was placed with the male overnight (Table II, 'Selective Mating System'). The statistical comparison of results with this mating system compared to standard mating was based on the total number of mice smeared, rather than the number mated, since the number of VP from the total colony was the logical basis for evaluating the effectiveness of the two methods.

Mating behavior was documented by observations on 4 clear plastic cages over a period

Table II. Effect of selective breeding on VP frequency in DES-exposed mice. Mating effectiveness is compared for 3-week periods starting when the mice were between 5 and 7 weeks of age.

Age weeks	Standard mating system		Selective mating system		Standard vs. selective
	Number of mice mated	Number of VP	Number of mice smeared	Number of VP	
5–7	108	5	32	3	$P > 0.5$
8–10	344	17	104	6	$P > 0.5$
11–13	476	3	292	13	$P < 0.001$
14–16	496	6	380	7	$P > 0.5$
17–19	72	1	160	4	$P > 0.5$

[a]Probability by χ^2 of differences in frequency of VP being due to chance.

of 45 min after introduction of a male to the female cage. Injury to the ears of the male was tabulated the next morning using the following scale: redness of the ears or periorbital area, or single small bite on the ear = 1; large bite = 2; large sore (multiple bites with ulceration) = 3; large number of overlapping bites that could not be accurately counted = 5.

To test for possible effects of age and vasectomy on acceptance of the male by females, an additional breeding experiment was performed. DES-exposed and VEH-exposed female mice of the same age were selected from the breeding colony. Both groups were first exposed to vasectomized mice overnight at 1-week intervals and then exposed to control mice overnight at 1-week intervals until termination of the experiment. During the latter matings, mice with VP were removed from the experiment and replaced by females of the same age. Ear damage was recorded as described above.

Results

The VP frequency in DES-exposed mice was lower than in VEH-exposed mice at all ages except the earliest and decreased more rapidly with age (Table I). Selective mating (single female selected by vaginal smear) did not improve the VP frequency except in the middle age group (Table II).

During 72 cage observations there was an average of 3.4 ± 0.46 (mean ± S.E.M.) hostile acts by each group of 4 DES-exposed females against the male. In contrast, 59 cage observation periods of VEH-exposed mice revealed an average of 0.9 ± 0.21 hostile acts against the male. This difference is significant at the $P < 0.001$ level. Injuries to vasectomized males housed overnight with DES-exposed females was significantly higher than to intact males housed overnight with VEH-exposed females not matched by age (Table III, columns 2 and 5). In the experiment designed to control for the variables of age and vasectomy, the DES-exposed females caused more damage to both the vasectomized males and the control males than did the VEH-exposed females (Table III, columns 3, 4 and 6, 7).

Discussion

The breeding performance of DES-exposed mice, as measured by VP frequency, was as strong as that of VEH-exposed mice at the earliest effective breeding age of 4 – 7 weeks. It then deteriorated rapidly. Since mice with VP were removed from the colony, some decrease in VP frequency could be proposed on the basis of selective removal of cyclic females. However, fewer DES-exposed females were removed at all time intervals

Table III. Comparison of injuries when a single control or vasectomised male was placed in a cage with four DES-exposed or VEH-exposed females.

Injury scale in points	Vasectomized males and			Control males and		
	DES[a]	DES	VEH	VEH[a]	DES	VEH
No injury	34	11	18	57	4	14
1 – 4	52	17	21	15	11	10
5 – 9	21	15	3	2	9	0
10 – 14	13	1	0	0	0	0
Over 14	1	0	0	0	0	0

[a]Females not controlled for age; females were matched by age in the remaining columns. All DES groups differed from corresponding VEH groups at $P < 0.001$ by analysis of variance.

except the first, so this effect would have biased the comparison with VEH-exposed females towards non-significance. The delayed anovulatory syndrome in rats produced by hormonal interference with hypothalamic sex differentiation was characterized by initial fertility followed by regression to infertility [4]. Thus, the decreasing fertility pattern seen with DES-exposed mice matches the anovulatory condition arising in rats from abnormal sex differentiation of the hypothalamus induced by exogenous hormones, including DES [1].

The phenomenon of lordosis, commonly used to evaluate female acceptance of the male in rats, was not seen in the mice. Nipping of the male's ear provides an alternative criterion for acceptance of the male by the female. By this criterion, DES-exposed females were significantly less accepting of the male than control females. Thus, the less receptive behavior of DES-exposed mice parallels that seen in rats with abnormal sex differentiation of the hypothalamus. A comparable tendency for reduced acceptance of the male by women exposed prenatally to DES was reported in the context of increased tendency to homosexuality [2].

The apparent cause of the delayed anovulatory syndrome is a decrease with time in the release of luteinizing hormone [4]. Such an effect was confirmed structurally by a deficiency of corpora lutea in DES-exposed mice [7]. This would be expected to alter endocrine function of the ovary. The issue is whether this hormonal imbalance could lead to cancer. Cancer in DES-exposed mice was prevented by ovariectomy, indicating dependence of the carcinogenic process on ovarian hormones [9]. The mechanism might be a promoting effect of hormones on changes initiated by exogenous carcinogens. Intravaginal administration of methylcholanthrene induced more vaginal tumors in mice exposed prenatally to DES than in control mice [10]. However, the resultant tumors were squamous cell carcinomas, not the adenocarcinomas characteristic of prenatal DES exposure. An alternative hypothesis is that DES induces somatic mutations, which is supported by evidence of DES genotoxicity [3]. Both factors might be operative and cancer would then be the product of hormonal imbalance promoting the induced mutations.

The importance of establishing whether DES causes cancer through altering sex differentiation of the hypothalamus is that other agents might have the same effect. For example, a high level of fat in the maternal diet has an effect similar to DES in that it increases the frequency of reproductive system tumors and pituitary tumors in the female offspring [12]. The mechanism of the dietary effect may also involve the estrogen-dependent sex differentiation of the hypothalamus, because fatty acids can bind to alpha-fetoprotein and thus have the potential to disrupt estrogen-dependent processes in the body [5]. Yet, concurrent production of somatic mutations by high levels of maternal dietary fat seems unlikely. Therefore, altered hormone balance may be a sufficient abnormality by itself to increase cancer frequency. If sex differentiation in the fetus is a sensitive process in respect to transplacental programming for cancer, then it becomes critically important to screen the various types of agents to which human fetuses are exposed for evidence of their ability to disrupt this differentiation process.

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