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LSD IN PREGNANCY: CHROMOSOMAL EFFECTS

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SUMMARY

The chromosomal effects of LSD in pregnant rats and their offspring were studied. Drug was given during implantation or during the period of differentiation and chromosome analyses were performed in the embryo and bone marrow cells from the mother and the adult offspring. Our results showed that exposure to pure LSD in pregnancy did not damage the chromosomes of the mother, embryos or adult offspring.

Considerable controversy exists in the literature regarding the teratogenic and mutagenic properties of lysergic acid diethylamide (LSD). The evidence that the drug produces embryonic malformations and chromosomal damage in human users and animals is inconsistent and equivocal (1-12). Under the present conditions, studies on human LSD users and their offspring do not appear to be a reliable experimental approach since the purity of the illicitly obtained drug as well as the amount and frequency of its ingestion are impossible to determine accurately. Furthermore, these subjects frequently experiment simultaneously with other psychotogenic drugs. It would seem therefore that reliable answers should come from well designed multispecies studies in experimental animals using LSD of established purity. This investigation forms part of a broader study (5,6) on the varied effects of exposure to LSD in pregnancy on the development of the offspring. In this report we describe the chromosomal effects of

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LSD on pregnant rats and their offspring.

Materials and Methods

LSD (Delysid, Sandoz, batch No. 88601) was obtained from the National Institute of Mental Health (IND 4184, Nair). The purity of the drug was verified by spectrophotofluorometric analysis (13). The drug was dissolved in isotonic saline and administered orally in a single dose of 100 μg/kg body weight to Sprague-Dawley rats on the 4th day of pregnancy (Group I) or on the 4th or 8th day of pregnancy (Group II). Rats receiving the vehicle only served as controls (Group III). The beginning of the first day of pregnancy was that morning when a positive vaginal smear for sperm was found in the mated female rat.

The rats of Group I were sacrificed on the 14th or 15th day of pregnancy and femur bone marrow cells were prepared for chromosome analysis by the direct method of Moorhead et al. (14). The embryos obtained from Group I rats were cultured *in vitro* for 12-14 days and the cells harvested for karyotyping, according to the method of Kadotani and Pergament (15). Rats of Groups II were allowed to deliver and raise their young; when the offspring were 8-15 weeks old, they were sacrificed and their femur bone marrow cells prepared for chromosome analysis similar to Group I animals. In all experiments pregnant animals and their offspring designated as controls (Group III) were paired with LSD-treated animals and subjected to the same conditions preparatory for chromosome analysis.

Slides from treated and control animals were coded to ensure that the examiner could not identify the source. Cells for analysis were selected with a low power objective (25X) and once a cell was selected, it was included in the total analysis. Analysis of each cell was carried out by visual examination under the microscope and included a chromosome count as well as evaluation for structural aberrations. Each cell was re-analyzed by a second examiner and each cell with a structural anomaly was photographed and confirmed by a third examiner. If there was no visible continuity of chromosomal structure and no displacement of alignment, these aberrations were scored as achromatic lesions. A break was characterized by displacement of the proximal end of the acentric fragment from a

position of alignment with the distal end of the centric chromosome. Chromatid and isochromatid breaks, acentric fragments and deletions were scored as single breaks; 2-break events included ring chromosomes, dicentrics, exchange configurations and translocations. Slides were not decoded until each cell had been counted and classified.

Significant differences existed within the LSD-exposed pregnant animals and within their progeny with respect to distribution of chromosome number and statistical analysis was based on paired, treated vs. untreated animals; for all other analysis the data were pooled. Although specific types of structural aberrations were listed individually, the numbers were too low for statistical analysis. Therefore, statistical analysis was based on the total number of structural chromosomal aberrations.

RESULTS

Following exposure to LSD, there was no statistically significant alteration in the distribution of chromosome number (aneuploidy vs. diploidy vs. polyploidy) and no significant increase in structural chromosomal aberrations in the cells of pregnant animals and their offspring. For Group I, the incidence of aneuploidy in 3 LSD-treated pregnant animals was 8.1, 8.2, and 20.2%, while in the matched, drug-free controls, 8.1, 16.7, and 23% respectively. A single tetraploid cell was seen in each member of a pair of animals (treated and control). The frequencies of total structural aberrations ranged from 0.0 to 1.86 per 100 cells for LSD-treated animals, 0.0 to 0.56 per 100 cells for control animals. The overall mean difference was not significant ($t = 0.86$; d.f.4; $P < 0.5$). (See Table 1).

The incidence of aneuploidy in cells of the LSD-exposed fetuses ranged from 24.4 to 28.3 percent, for the untreated fetuses, from 22.4 to 32 percent. Polyploidy varied from 1.6 to 13.2 percent for the drug-exposed fetuses and from 3.7 to 5 percent for its drug-free control. The frequencies of total structural aberrations ranged from 1.2 to 6.6 percent for fetuses exposed to LSD *in utero* and

from 2.4 to 4.9 percent for control fetuses. The overall mean difference was not significant (t=0.302; d.f.4; P<0.7). (See Table).

TABLE 1

Structural Chromosome aberrations in Pregnant rats and their offspring exposed to LSD

Group	No. of Animals	No. of Cells	Structural aberrations (mean/100 cells) 1 Break	2 Break	Achromatic lesions
Pregnant rats (Gr. I)					
LSD-exposed	3	611	0.61	-	0.37
Control	3	424	0.28	-	0.28
Fetuses (Gr. I)					
LSD-exposed	28	517	1.99	-	1.64
Control	22	633	1.48	0.22	2.42
Offspring (Gr. II)					
LSD at 4 days	2	106	-	-	0.39
Control	2	101	-	-	1.92
Offspring (Gr. II)					
LSD at 8 days	2	102	1.6	-	1.25
Control	2	78	1.4	-	-

The mean incidence of aneuploidy in marrow cells of progeny exposed to LSD in utero at 4 or 8 days gestation (Group II) was the same, 13.6%; for the matched controls, 8.9 and 11.7% respectively. A single polyploid cell was observed in one control animal. The frequency of structural aberrations was 0.0 and 0.78% for the offspring exposed to LSD at 4 days gestation, 0.0 and 3.8% for controls; the difference was not significant (t= .550, d.f.2; P<0.7). For the offspring exposed to LSD at 8 days gestation, the frequency of structural aberrations was 2.5 and 3.2% while for the controls, 0.0 and 2.8 percent, again; the difference was not significant. (t=0.417; d.f.2; P<0.7).

DISCUSSION

This study was concerned with the possible effects on the integrity of rat

chromosomes when exposed to LSD in utero, specifically at the time of implantation (4-5 days post-fertilization) and rapid cell division and differentiation (8 days post-fertilization). In addition, an attempt was made to determine if the effects of LSD on chromosome integrity was short and/or long term. We were unable to demonstrate any significant chromosomal effect from in utero exposure to LSD, although there is evidence that LSD crosses the placenta and is present in fetal tissues in detectable amounts up to 4 hours after administration (16).

The present authors also had an opportunity to test the peripheral leukocytes of two college students - one, a male and the other a pregnant female. Both have claimed to have taken LSD several times, the female having taken the drug while pregnant. In both cases, chromosomal analysis revealed no abnormalities. This female delivered an apparently normal healthy baby. Earlier, Sato and Pergament (17) studied the chromosomes of a newborn baby girl whose mother had taken 4 doses of LSD before and 2 doses during pregnancy. (43rd and 57th day after her last menstrual period). No significant increase in chromosomal aberrations or the presence of any congenital malformations was observed even though LSD was taken during the critical period for the production of leg deformities.

The negative results of the present study with pure LSD are in agreement with the more recent observations of other investigators in humans (18-21) and experimental animals (4, 22-26), including drosophila (23), mice (22, 24, 26), hamsters (22, 26), rats (4, 26), rabbits (24). Nevertheless, we cannot ignore the positive findings of teratogenic and mutagenic effects of LSD reported by some investigators. The factors underlying these differing responses may be complex and may include, such things as strain differences, individual threshold differences, genetic susceptibility, manner of drug administration, co-existing subclinical viral infections, purity of the drug and exposure to other drugs or chemicals at the same time as LSD. The last two are particularly of relevance in the case of human subjects, for there are at least two in vivo studies where no chromosomal damage was observed following administration of pure LSD (19,20).

In a recent article (27) reviewing the available evidence for implicating LSD in chromosomal or genetic damage, Dishotsky et al. have concluded that pure LSD ingested in moderate doses does not damage chromosomes in vivo.

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