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THE MUTAGENIC EFFECT OF LYSERGIC ACID DIETHYLAMIDE
I. CYTOGENETIC ANALYSIS

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SUMMARY

The effect of lysergic acid diethylamide (LSD) was studied in rats and mice by analysing chromosomes of primary spermatocytes.

Male Wistar rats received a single dose of LSD (10 or 1000 μg/kg b.w.). The spermatocytes in diakinesis/ first metaphase were analysed on day 1, 7, 21 and 41 after the administration.

ICR male mice were treated by a dose of 1000 μg LSD/kg b.w. A testicular analysis was made after 12 weeks.

Chromosomal breaks were sporadic and non-significant, with no chromosomal rearrangements. A significant increase of spermatocytes, with univalents, in comparison with controls, was found in all experimental groups. Rats showed a distinct increase in the occurrence of spermatocytes with autosomal univalents; in mice there was an increased frequency of autosomal as well as X–Y univalents.

INTRODUCTION

Numerous papers have been published in recent years attempting to contribute to knowledge on the mutagenic effect of LSD in various organisms. The conclusions of individual authors are contradictory, especially as to the ability of LSD to induce chromosomal aberrations[4,9].

The possible effects on the offspring of individuals using LSD have also given an impulse to the study of chromosomal changes in meiotic cells. An increased frequency[3] of chromosomal breaks in spermatocytes of mice given doses of 25 μg LSD/kg b.w. and 1000 μg LSD/kg b.w.[12] has been reported. JAGIELLO AND POLANI[7], however, found no structural aberrations in mice, either after a single dose of 1000 μg LSD/kg b.w., or after a chronic administration (31 days) with total doses of 500, 1000, 2500 or 5000 μg/LSD/kg b.w. EGOZCUE AND IRWIN[5] also published negative findings of chromosomal aberrations in the spermatocytes of mice and *Macacus mulattus* after a

Abbreviation: LSD, lysergic acid diethylamide.

single dose as well as after chronic administration of LSD. HULTEN *et al.*[6], using LSD, found negative results on the bioptic testicular material from two men.

In our experiments we have studied the influence of LSD on meiotic chromosomes in dependence on the dose and stage of spermatogenesis.

MATERIALS AND METHODS

Two groups of male Wistar rats were treated intraperitoneally with a single dose of 10 μg LSD/kg b.w. or 1000 μg LSD*/kg b.w. Testicular analyses were made on days 1, 7, 21 and 41 after the administration in order to examine the diakinesis/first metaphase of various types of cell directly affected by the drug. The experimental animal groups were marked according to the dose applied (A, 10 μg LSD/kg b.w.; B, 1000 μg LSD/kg b.w.) and according to the time of chromosomal examination after the application (A1-4 and B1–4 were examined after 1, 7, 21 and 41 days). The testes for the analyses of chromosomal abnormalities were obtained subsequently by semicastration so that two samples were taken from an individual animal at various times. In each experimental group consisting of 6 animals we analysed a total of 600 spermatocytes, always 100 spermatocytes from one testis of each animal. 6 animals were used as controls in which 1200 diakinesis/first metaphases were analysed.

10 male mice of ICR strain were given a single dose of 1000 μg LSD/kg b.w. Testicular analyses were made 12 weeks after application. In each male 200 diakinesis/first metaphases from both testes were analysed.

The testicular material was treated according to the modified MEREDITH method[10].

Chromosomal rearrangement as well as the presence of autosomal and X–Y univalents were estimated in the spermatocytes. In the group of spermatocytes with univalents, we included the cells in which the univalents occurred in distinctly remote sites of the diakinetic figure.

RESULTS

Rats

Chromosomal breaks were sporadic; in no experimental group did they exceed the control values. No chromosomal rearrangement was found.

In spermatocytes with univalents, unpaired chromosomes but no chromosomal fragments were found. In all experimental, as well as in control groups, autosomal univalents were distinctly predominant. The distribution of unpaired bivalents occurred according to a random pattern.

A statistical evaluation (χ^2 test) of the frequency of spermatocytes with univalents between experimental and control groups revealed a significant difference at the 1% level in groups A_1, A_2, A_4, B_1, B_2, B_3 and B_4; group A_3 showed a difference at the 5% level of significance (Table I).

Mice

Breaks were sporadic and non-significant and no chromosomal rearrangements were found.

* LSD, Lysergamid, Spofa, Czechoslovakia. (D-Lysergic acid diethylamide tartrate, Batch No. 040 168.)

There was a significant difference (χ^2 test) at the 1% level between the frequency of spermatocytes with univalents in both the experimental and control groups (Table II).

DISCUSSION

We found no gross morphological changes in the chromosomes of male rat and mouse germinal cells influenced with LSD. These conclusions agree with the findings of JAGIELLO AND POLANI[7] in mice, EGOZCUE AND IRWIN[5] in mice and *Macacus mulattus* and with results obtained by HULTEN *et al.*[6] in men. The studies describing an increased frequency of chromosomal breaks, gaps and secondary constrictions in mice[3,12] were carried out on only a small number of animals. According to our view the contemporarily applied cytological methods analysing testicular tissues are not sufficiently refined to permit an exact evaluation of gaps and secondary constrictions. Moreover, the significance of gaps and secondary constrictions in relation to mutagenesis is still questionable. SKAKKEBAEK *et al.*[12] found in mice 3.6% aberrations of chromosomal morphology (breaks, gaps, secondary constrictions) after the application of 1000 μg LSD/kg b.w. The percentage of the breaks themselves was small—0.82 to 0.98% even if we take into account that there were no breaks in the control group.

Our experiments showed an increased frequency of spermatocytes with univalents in rats given a high as well as a low dose of LSD and examined 1, 7, 21 and 41

TABLE I

ANALYSIS OF RAT SPERMATOCYTES IN DIAKINESIS/FIRST METAPHASE

Group of animals	*N*	*Normal cells*		*Cell with univalents*					
		abs	%	*Autosomal*		*X/Y univalents*		*Total*	
				abs	%	*abs*	%	*abs*	%
A_1	600	520	86.7	78	13.0	2	0.3	80	13.3
B_1	600	494	82.3	103	17.2	3	0.5	106	17.7
A_2	600	525	87.5	75	12.5	–	–	75	12.5
B_2	600	518	86.3	80	13.4	2	0.3	82	13.7
A_3	600	536	89.3	63	10.5	1	0.2	64	10.7
B_3	600	524	87.3	74	12.4	2	0.3	76	12.7
A_4	600	725	87.5	75	12.5	–	–	75	12 5
B_4	600	506	84.3	91	15.2	3	0.5	94	15.7
Control	1200	1106	92.2	78	6.5	16	1.3	94	7.8

A, groups of animals treated with 10 μg LSD/kg b.w.
B, groups of animals treated with 1000 μg LSD/kg b.w.
1, 2, 3, 4, groups of animals analysed 1, 7, 21 and 41 days after treatment.
N, number of cells analysed.

TABLE II

ANALYSIS OF MICE SPERMATOCYTES IN DIAKINESIS/FIRST METAPHASE

Group of animals	*N*	*Normal cells*		*Cells with univalents*					
		abs	%	*Autosomal*		*X/Y univalents*		*Total*	
				abs	%	*abs*	%	*abs*	%
1000 μg LSD/kg b.w.	2000	1937	96.85	26	1.30	·37	1.85	63	3.15
Control	2000	1987	99.35	5	0.25	8	0.40	13	0.65

N, number of cells analysed.

days after the administration. In mice that received 1000 μg LSD/kg b.w. the frequency of spermatocytes with univalents was also significantly increased 84 days after the administration. Whereas in the influenced as well as in the control mice X–Y univalents predominated, which is in agreement with the literature data, in rats the autosomal univalents predominated. This finding might be attributed to a species-dependent occurrence of autosomal and X–Y univalents.

The considerable individual, strain- and species-dependent variability in the frequency of spermatocytes with univalents, even in untreated animals[11], diminishes the significance of this finding for the assessment of the mutagenic effects of chemical agents. We cannot say whether this is due to asynapsis or desynapsis. Similarly the effect of the method is not clear[8].

Nonetheless, SCHLEIERMACHER[11] described an increased proportion of spermatocytes with univalents in mice 6 to 31 and 4 to 22 days after the administration of Trenimon and Endoxan respectively. On the other hand, LÉONARD AND LINDEN[8] found no significant increase of univalents 100 days after the application of six monofunctional and poly-functional alkylating agents. Neither did ADLER AND RÖHRBORN[1] after a chronic treatment with caffeine.

The pathophysiological processes responsible for the onset of univalents are still obscure. There is no proof that the presence of univalents could be attributed to small structural chromosomal aberrations[11]. One possible explanation might be the mutation of genes controlling actual cell division, specifically the synapsis and desynapsis of homologous chromosomes. Impaired cell division of germ cells characterized by chromosome losses in *Drosophila melanogaster* after the treatment of LSD was described by BROWNING[2] and by ŠRÁM[14].

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