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Endocrine and Metabolic Effects of Lysergic Acid Diethylamide on Female Rats

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The psychic symptoms caused by minute doses of lysergic acid diethylamide (LSD) resemble certain aspects of the schizophrenic process (Forrer and Goldner, 1951; Lingjaerde and Skaug, 1956). The hallucinogenic drug has been used to explore the induction of reversible psychoses in nonpsychotic subjects (Forrer and Goldner, 1951). It has been assumed that the psychomimetic effects produced by LSD could yield information pertinent to the mechanisms and pathophysiology involved in the schizophrenias (Lingjaerde and Skaug, 1956; A. M. Sackler *et al.*, 1954).

In general, the literature contains contradictions and inconsistencies concerning the effects of LSD on white blood cells (Forrer and Goldner, 1951; Belsanti, 1952; Rothlin *et al.*, 1956; Feld *et al.*, 1958; Härkonen and Könttinen, 1958) and the endocrine system of laboratory animals and man (Bliss *et al.*, 1956; Kar and Boscott, 1956; Lingjaerde and Skaug, 1956; Nadel *et al.*, 1957; Jovanovic *et al.*, 1960). Investigations with male Wistar rats have indicated that LSD stimulated adrenal function, significantly lowered body weights, food consumption, and metabolism rates, and inhibited thyroidal and possibly gonadal function (Weltman and Sackler, 1966). This investigation sought to determine the physiologic responsiveness of female Wistar rats of the same strain to comparable doses of LSD on body growth, white blood cells, and endocrine function.

METHODS

Forty-eight female Wistar rats, averaging 100 g in body weight were distributed into three equal groups. Animals in groups A and B received a series of subcutaneous injections of LSD.[1] The doses consisted of 500 or 750 μg/kg dissolved in 1 ml of 0.9% NaCl solution. Group C, the control rats, received an equivalent amount of saline solution. These doses were used in view of reports that rats and mice require more than 1000 times the effective psychomimetic dose in man to produce autonomic and behavioral responses (Nadel *et al.*, 1957). To observe the influence of duration of treatment, eight injections were administered during a 2-week period on alternate days with the exception that both experimental groups were injected on days 13 and 14. The procedure of alternate days of injection was selected to minimize the development of resistance to prolonged administration of the drug. Mahler and

[1] Sandoz Pharmaceuticals, Hanover, New Jersey.

Humoller (1959) reported that rats became refractory after several injections to doses of LSD ranging from 300 to 700 μg/kg.

To avoid the chance of increased fighting and injuries following the administration of LSD, all animals were housed singly in metal cages in air-conditioned quarters maintained at 73°F. It is recognized, however, that acute and chronic isolation per se can produce complicating behavioral, metabolic and endocrinologic alterations (Weltman *et al.*, 1962).

Body weight and food consumption were determined weekly during the 2-week experimental period. Blood specimens were taken from each animal by oblique incisions of the tail vein at the end of the 2-week experimental period immediately prior to autopsy. Standard techniques were used for total leukocytes (Todd and Sanford, 1944) and eosinophil counts (Henneman *et al.*, 1949). The differential blood slides were stained with MacNeal tetrachrome solution (Gurr, 1956), and analyses were based on counts of 200 cells per animal.

All test and control rats were sacrificed by etherization and autopsied at the conclusion of the 2-week study. The endocrine and associated accessory organs were weighed to the nearest 0.1 mg (thymus, adrenal, ovary, uterus, and thyroid). The tissues were preserved in fixative,[2] sectioned at 5μ, and stained with hematoxylin and eosin. The results of the histologic findings will be reported in a separate communication.

RESULTS

Analyses of the body weight data by standard *t*-test procedures (Snedecor, 1949) indicated that neither of the two dosages caused significant alterations in the body weight of the female rats. At the end of the second week of drug administration, the 500 and 750 μg/kg dosages caused reductions of 2.5% and 2.7% in the body weights of the group A and B animals, respectively.

TABLE 1
EFFECT OF SUBCUTANEOUS ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE ON FOOD CONSUMPTION OF FEMALE RATS

Group	Dosage (μg/kg)	Number of animals	Food consumed (g)[a]		
			1st Week	2nd Week	Total
Group A	500	16	81.4 ± 2.5	87.3 ± 2.7	168.7 ± 4.5
Group B	750	16	78.5 ± 1.6	84.7 ± 3.1	163.2 ± 3.6
Group C	Control (saline)	16	87.3 ± 2.1	90.1 ± 2.5	177.4 ± 3.9
P value, A *vs.* C	—	—	0.09	0.44	0.16
P value, B *vs.* C	—	—	<0.01	0.19	0.01
P value, A *vs.* B	—	—	0.34	0.52	0.35

[a] Mean ± SE.

Table 1 presents the food consumption of the test and control groups. The results indicated that the 750 μg/kg dose caused significant decreases in food consumption during the first week and for the combined 2-week period. The 6.0% decrease in food

[2] Fixative FU-48, Technicon Instruments Corp., Chauncey, New York.

TABLE 2

EFFECT OF SUBCUTANEOUS ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE ON TOTAL LEUKOCYTE AND EOSINOPHIL FREQUENCIES AND DIFFERENTIAL WHITE BLOOD CELL COUNTS IN FEMALE RATS

Group	Dosage (μg/kg)	Number of animals	Leukocytes[a] (cells/mm³)	Eosinophils[a] (cells/mm³)	Differential absolute counts		
					Lymph./mm³	Neutro./mm³	Eosin./mm³
Group A	500	16	12,934.4 ± 830.3	65.6 ± 8.2	10,329.9 ± 640.5	2,462.1 ± 219.2	43.6 ± 12.4
Group B	750	16	11,643.8 ± 880.5	66.9 ± 10.6	9,511.9 ± 711.1	1,990.2 ± 175.2	51.3 ± 18.0
Group C	Control (saline)	16	15,228.1 ± 1,029.9	100.0 ± 13.7	12,650.5 ± 916.1	2,424.1 ± 247.2	75.1 ± 17.9
P value, A *vs.* C	—	—	0.09	0.04	0.05	>0.90	0.16
P value, B *vs.* C	—	—	0.01	0.07	0.01	0.17	0.36
P value, A *vs.* B	—	—	0.30	>0.90	0.40	0.10	0.73

[a] These are wet-chamber counts performed on blood taken immediately before sacrifice. The mean ± SE is given.

TABLE 3

EFFECT OF SUBCUTANEOUS ADMINISTRATION OF LYSERGIC ACID DIETHYLAMIDE ON ABSOLUTE ENDOCRINE AND ASSOCIATED ORGAN WEIGHTS OF FEMALE RATS

Group	Dosage (μg/kg)	Number of animals	Final body weight (g)[a]	Thymus (mg)[a]	Adrenals (mg)[a]	Ovaries (mg)[a]	Uterus (mg)[a]	Thyroid (mg)[a]
Group A	500	16	123.1 ± 3.1	191.4 ± 12.7	33.2 ± 1.7	39.8 ± 2.6	162.5 ± 20.2	9.9 ± 0.4
Group B	750	16	122.9 ± 2.3	198.6 ± 13.8	31.3 ± 1.3	37.0 ± 2.5	156.0 ± 16.3	9.4 ± 0.3
Group C	Control (saline)	16	126.3 ± 2.0	250.5 ± 10.9	31.4 ± 0.9	37.6 ± 1.8	186.5 ± 12.8	10.3 ± 0.4
P value, A *vs.* C	—	—	0.40	<0.01	0.35	0.49	0.33	0.49
P value, B *vs.* C	—	—	0.28	<0.01	>0.90	0.85	0.16	0.10
P value, A *vs.* B	—	—	0.61	0.70	0.67	0.45	0.80	0.34

[a] Mean ± SE.

intake observed during the second week was not statistically significant. Similar, but lesser reductions in food consumption were noted in animals given 500 μg/kg. The most marked decrease, 6.8%, induced by the smaller dose occurred during the first week, but was not statistically significant ($P = 0.09$). Although no significant differences were observed between the two dosages, the respective decreases in food intake suggested a dosage relationship.

In accord with food consumption alterations, examination of the body weight gain findings revealed that the greatest decreases in the weight gains of the test rats as compared to the controls occurred during the first week (group A, 23.2%; group B, 17.2%). However, none of the body weight gain alterations were statistically significant.

Table 2 lists the total white blood cell and eosinophil counts obtained by the chamber method, and the absolute values of the lymphocytes, neutrophils, and eosinophils as determined from the differential blood slides. The chamber counts demonstrated that both doses of LSD induced marked or significant decreases in the total leukocyte and eosinophil cells of the test rats at the completion of the 2-week administration period. In all cases, the P values were either less than 0.05 or approached the 0.05 level of significance.

Although analyses of the relative frequencies of the differential white blood cells indicated trends of lymphopenia, eosinopenia, and neutrophilia, calculations revealed significant decreases in the absolute number of lymphocytes but no evidence of neutrophilia. The percentage decreases in the absolute eosinophil counts derived from the differential blood slides corresponded to the reductions observed by the wet-chamber method. The discrepancies between the P values of the two procedures can be attributed, however, to the fact that the differential method is a much less accurate procedure for estimating eosinophil counts (Randolph and Stanton, 1945).

Table 3 presents the final body weights and the absolute organ weights of the endocrine and associated organs of the groups sacrificed at the end of the 2-week period. As stated previously, evaluation of the body weight data indicated moderate reductions in the test animals which were not statistically significant. Both doses induced significant decreases in the thymus weights of the test rats. No significant changes were observed in the absolute adrenal weights of the test groups. On a relative weight basis, group A animals presented the greatest increase in the adrenal weights which although showing a low P value did not attain statistical levels of significance ($P = 0.11$). Consistent but nonsignificant decreases were also noted in the uterine and thyroid weights of both groups. In the case of the absolute thyroid weights at the higher dosage, the reduction approached statistical levels of probability ($P = 0.10$).

DISCUSSION

In evaluating the effects produced by LSD in female rats, the various findings, although less pronounced, paralleled results observed in treated male rats (Weltman and Sackler, 1966). It is apparent from the marked or significant decreases in total leukocytes, lymphocytes, eosinophils, and thymus weights that administration of LSD to female Wistar rats caused increased adrenocorticosteroid output. Reductions in total leukocytes, lymphocytes, and eosinophils and involution of the thymus have been used as parameters of adrenocortical function. The white blood cell data consequently

agree with other investigations reporting decreased lymphocytes (Belsanti, 1952) and eosinophils (Härkonen and Könttinen, 1958; Feld *et al.*, 1958; Belsanti, 1952) by administration of LSD. More pronounced and significant decreases were observed in the total leukocyte and eosinophil counts of the equivalently treated male Wistar rats (Weltman and Sackler, 1966).

Paradoxically, the small or moderate alterations found in the absolute adrenal weights of the test rats did not correspond to the marked or significant changes in the white blood cell counts and thymus organ weight data. The counterpart investigations on males demonstrated marked and significant increases in the absolute adrenal weights and significant increases in the relative adrenal weights of both test groups. Conceivably, the smaller changes in adrenal weights of females could signify either changes leading to adrenal exhaustion, drug tolerance, the unresponsiveness of adrenals to continuous administration of LSD, or greater resistance of the females to nonspecific stress situations. It has been demonstrated that adrenal weights will decrease in individuals exposed to intense continuous stress (Uotila and Pekkarinen, 1951). Similarly, various investigators have reported that daily administration of LSD produced rapid development of tolerance in the physiologic and behavioral responses of man (Balestrieri and Fontanari, 1959) and laboratory animals (Mahler and Humoller, 1959). Rinkel *et al.* (1954) also reported that while LSD stimulated the "pituitary-adrenal axis" it made the adrenals somewhat unresponsive to adrenocorticotropin. In the present investigation, however, the premise of greater resistance capacities of female rats to nonspecific stress appears more plausible. Previous vibration stress studies with male and female rats of the same strain and age also demonstrated greater decreases in body weight and food consumption, and significant adrenal hypertrophy in the treated males (A. M. Sackler and Weltman, 1966). Histologic examination of the adrenals may help to better define the issue.

Trends indicative of reduced thyroid function stemmed from the decreases noted in the food consumption and thyroid weights of the test animals, especially in group B. The comparable investigation of males with the 500 and 750 μg/kg doses demonstrated not only more marked and significant decreases in food consumption, body weight, and thyroid weight, but also marked or significant decreases in the O_2 consumption rate of the test males.

Similar suggestions of reduced gonadal function arise from the consistent trends of marked decreases observed in the uterine weights of the test animals with greater effects being produced by the higher dose. Again, the comparable studies with male rats revealed still more pronounced weight decreases in the accessory reproductive organs of the males, i.e., seminal vesicles.

In conclusion, despite moderating effects due probably to sex-related resistance factors, the findings in the present study of females agree with the previously reported investigation of males. It is of interest that while it is dangerous to relate behavioral and endocrine alterations in laboratory animals to man, the results suggest a possible parallelism of certain LSD intoxication responses to those noted in schizophrenia. Condrau (1949) has advanced the concept that psychoses may be the result of endogenously produced substances similar to LSD. Rinkel *et al.* (1955) have postulated that LSD may interfere with adrenaline metabolism, yielding noxious and deleterious metabolites; and Lingjaerde and Skaug (1956) have claimed that dis-

turbances in carbohydrate metabolism found in schizophrenics may be similar to those produced by LSD. Increased output of adrenocorticosteroids has been reported in schizophrenic patients (M. D. Sackler *et al.*, 1950; Bliss *et al.*, 1956; Altschule, 1957). Conditions of hypothyroidism (A. M. Sackler *et al.*, 1951; Reiss *et al.*, 1952) and hypogonadism (M. D. Sackler *et al.*, 1952) have also been associated with this psychiatric disorder. Lowered eosinophil levels (M. D. Sackler *et al.*, 1951; Altschule, 1957), heightened 17-ketosteroid output (Altschule, 1957), and increased 17-hydroxycorticosteroid secretion (Bliss *et al.*, 1956) have been similarly demonstrated in disturbed schizophrenics. In general, the pattern of the findings caused by administration of LSD to female and male rats parallels the endocrine status observed in schizophrenics. Thus, the present findings support the concept that increased adrenocortical function together with relative or absolute hypothyroidism or hypogonadism may be associated with etiologic and pathogenic factors in some of the schizophrenias (A. M. Sackler *et al.*, 1954).

SUMMARY

The effects of the acute and prolonged administration of the hallucinogenic drug lysergic acid diethylamide (LSD) on white blood cell counts, body weight, food consumption, and weight of the thymus, adrenal, ovary, uterus, and thyroid of female Wistar rats were investigated. Test animals were given eight subcutaneous injections containing 500 or 750 μg of LSD per kilogram dissolved in 1 ml of saline. The eight injections were administered on alternate days during a 2-week period with the exception that both experimental groups received injections on days 13 and 14. Tail blood samples were taken immediately prior to autopsy. Marked or significant decreases were noted in total white blood cell counts as well as lymphocyte and eosinophil frequencies. Food consumption was affected more adversely during the first week. Analyses of the absolute organ weight data revealed significant reductions in the thymus weights of both test groups, with consistent but smaller decreases in the thyroid and uterine weights of the test animals. Although administration of LSD to female Wistar rats demonstrated greater resistance and less pronounced effects than those noted in males, the results still indicated stimulated adrenal activity with suggestions of hypothyroidism and hypogonadal function.

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