Endocrine Effects of Chronic Administration of Psychoactive Drugs to Prepubertal Male Rats. II. LSD[1]

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The endocrine effects of chronic D-lysergic acid diethylamide (LSD) administration to prepubertal animals were studied by injecting intraperitoneally three times a week for a month either 100 μg or 500 μg of the psychoactive drug per kilogram or the vehicle to groups of Sprague–Dawley male rats starting at 21 days of age. Animals injected with either dosage of LSD had smaller body weights than controls and tail length was significantly reduced in the high dosage group. plasma levels of growth hormone (GH) were decreased in the high dosage group, and pituitary levels in the low dosage group. Plasma levels and pituitary concentrations of luteinizing hormone and follicle stimulating hormone were not significantly modified by the drug. The low dosage of LSD decreased the brain levels of noradrenaline and increased those of dopamine, while the high dosage decreased those of 5-hydroxyindoleacetic acid. These data suggest that LSD, when administered chronically to developing animals, can inhibit body growth probably by altering the secretion of GH through modifications of its neuroendocrine control.

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Les effets endocriniens de l'administration chronique d'acide D-lysergique diethylamide (LSD) à des animaux prépubères furent étudiés en injectant par voie intrapéritonéale trois fois par semaine pendant un mois 100 ou 500 μg de la drogue par kilogramme ou son véhicule à des groupes de rats mâles Sprague–Dawley à partir du 21e jour de vie. Les animaux injectés avec du LSD avaient des poids corporels plus petits que ceux des contrôles, et la longueur de la queue était réduite chez les rats ayant reçu la dose élevée. Les niveaux plasmatiques d'hormone de croissance (GH) étaient abaissés dans le groupe ayant reçu la dose forte et les niveaux hypophysaires dans celui ayant reçu la dose faible. Les niveaux plasmatiques et hypophysaires d'hormone lutéinisante et folliculo-stimulante ne furent pas modifiés de façon significative par le LSD. La dose faible de la drogue abaissa les taux cérébraux de noradrénaline et entraîna une augmentation des taux de dopamine, et ceux de l'acide 5-hydroxyindoleacétique furent abaissés par la dose forte. Ces résultats suggèrent que le LSD, lorsqu'il est administré de façon chronique à des animaux en voie de développement, peut inhiber la croissance corporelle en altérant probablement les mécanismes neuroendocriniens régulateurs de la sécrétion du GH.

Introduction

Several reports have indicated that the acute or chronic administration of LSD[2] to animals can modify the concentration and/or the turnover of some of the biogenic amines of the central nervous system (for a review see: Leonard 1972). It is well known, on the other hand, that the biogenic amines are involved in the control of the secretion of several anterior pituitary hormones (for a review see: Collu *et al.* 1973*a*). It is surprising therefore that no reports have appeared to our knowledge on the endocrine effects of the chronic administration of this psychoactive drug. The present work was undertaken to study the endocrine effects of long term administration of LSD to prepubertal male rats.

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[2]The following abbreviations are used: LSD, D-lysergic acid diethylamide; GH, growth hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; NA, noradrenaline; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; Δ^9-THC, Δ^9-tetrahydrocannabinol.

Materials and Methods

Experiment 1

Groups of 21-day-old male Sprague–Dawley rats were injected intraperitoneally three times a week for

TABLE 1. Body weight, tail length, and endocrine organ weights of male rats injected intraperitoneally three times a week for a month with LSD or vehicle (treatment started at 21 days of age)

Group[a]	Body wt. (b.w.), g	Tail, cm	Pituitary	Brain	Adrenals	Prostate	Seminal vesicles	Testes
			mg/100 g b.w.					
LSD, 100 μg/kg (10)	219±7[b,c]	18.2±0.2	2.8±0.1	607±17	16.1±0.6	90±1	102±6	1086±34
Vehicle (10)	239±6	18.0±0.2	3.0±0.1	554±23	16.1±0.6	84±6	94±7	1021±46
LSD, 500 μg/kg (10)	212±4[c]	15.8±0.2[d]	3.0±0.1	604±19	16.4±1.4	87±5	90±3	1050±20
Vehicle (10)	228±5	16.9±0.2	3.0±0.1	582±14	15.1±0.4	89±5	91±5	1090±20

[a]Number of rats in parentheses.
[b]Values are means ± SE.
[c]$P < 0.05$ *versus* vehicle.
[d]$P < 0.01$ *versus* vehicle.

a month with either 100 μg of LSD[2] per kilogram or with saline (1 ml/kg). An intermittent schedule of administration was chosen to prevent the development of tolerance. LSD was obtained from Health and Welfare Canada, Ottawa, Canada. The animals had free access to water and rat pellets, but the amount of food ingested was recorded daily. The housing quarters had a constant temperature of 22 °C and a light–dark cycle of 14–10 h starting at 0500 hours. At the end of the experiment the rats were sacrificed by decapitation between 1000 and 1200 hours to avoid variability due to the diurnal fluctuations of plasma GH levels (Collu *et al.* 1973*b*). Rats were decapitated 30–45 min after the last injection since peak changes in monoamine levels are reported to occur at that moment. Handling of the animals was limited to a minimum, and decapitations were performed in a room separate from living quarters within 2 min after removal from the cages. Body weight, tail length, and the weights of brain, anterior pituitary, adrenals, testes, prostate, and seminal vesicles were recorded. Trunk blood was collected in heparinized tubes. Plasma were then separated and stored at −20 °C until determination of GH levels by the radioimmunoassay method of Schalch and Reichlin (1966), LH levels by the radioimmunoassay method of Niswender *et al.* (1968) with NIAMD-Rat-LH-RP1[3] as standard preparation, and FSH levels by the radioimmunoassay method of Parlow *et al.* (1969) with NIAMD-Rat-FSH-RP1 as standard preparation. Anterior pituitaries were homogenized in 5 ml of saline, and the supernatant kept at −20 °C until determination of GH, LH, and FSH levels. All hormone determinations were performed in single batches. The brains were stored at −20 °C for no more than 2 weeks until determination of the levels of 5-HT, NA, and DA. This was performed in the same sample, after extraction in acidified butanol by the method of Ansell and Beeson (1967) for NA and DA. Fluorescence values were read in an Aminco–Bowman spectrophotofluorometer.

Experiment 2

In this experiment, which was performed 2 months later, the protocol of experiment 1 was followed with some modifications. The amount of LSD was increased to 500 μg/kg. This was done because high doses have been reported to produce biochemical changes different from those obtained with lower doses (Peters 1974). Brain levels of 5-HIAA were also measured in the same brain sample, in addition to those of 5-HT, NA, and DA, by the method of Miller *et al.* (1970), to obtain some information on the turnover of 5-HT.

Statistical Analysis

Statistical evaluation of the data was made with the Student *t* test.

Results

No grossly evident behavioral modifications followed the administration of either dosage of LSD.[2] Food intake remained unchanged in the low dosage group, but was slightly reduced in the high dosage group (controls, 15 g per rat per day; LSD, 13 g per rat per day). The effects on body weight, tail length, and organ weights are shown in Table 1. Both LSD dosages significantly reduced body weight, but only the high dosage significantly reduced tail length. Organ weights, expressed as percentage of body weight, were not modified by the drug although the absolute weight of some organs was reduced in the high dosage group. As shown in Fig. 1, plasma levels of GH were significantly depressed by the high dosage. Pituitary GH content and concentration were significantly reduced by the low dosage. This could indicate an inhibition of GH secretion by the low dosage and of GH synthesis by the high dosage. Plasma levels of LH were not modified by LSD, as shown in Fig. 2. Pituitary content, but not concentration, was reduced by the low dosage. Plasma and pituitary levels of FSH were not modified by the drug, as shown in Fig. 3. The effects of LSD on the brain levels

[3]NIAMD, National Institute of Arthritis, Metabolism and Digestive Diseases.

TABLE. 2. Brain content of 5-HIAA, 5-HT, NA, and DA in male rats injected intraperitoneally three times a week for a month with LSD or vehicle (treatment started at 21 days of age)

Group[a]	5-HIAA	5-HT	NA	DA
	ng/g			
LSD, 100 μg/kg (10)	—	658 ± 132[b]	412 ± 24[c]	1641 ± 71[d]
Vehicle (10)	—	652 ± 62	505 ± 18	1264 ± 52
LSD, 500 μg/kg (10)	418 ± 16[e]	652 ± 21	387 ± 6	1011 ± 31
Vehicle (10)	528 ± 35	590 ± 21	395 ± 9	976 ± 33

[a]Number of rats in parentheses.
[b]Values are means ± SE.
[c]$P < 0.01$ *versus* vehicle.
[d]$P < 0.001$ *versus* vehicle.
[e]$P < 0.02$ *versus* vehicle.

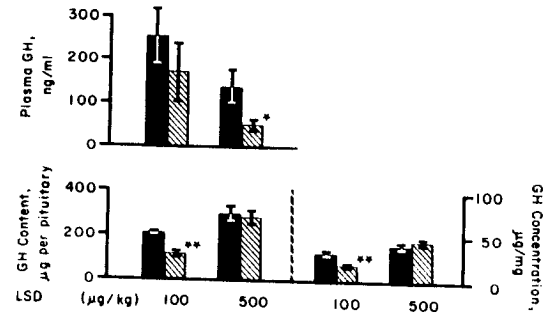

FIG. 1. Effect of chronic intraperitoneal injections of LSD on plasma and pituitary levels of GH. ■, vehicle; ▩, LSD; *, $P < 0.02$; **, $P < 0.001$.

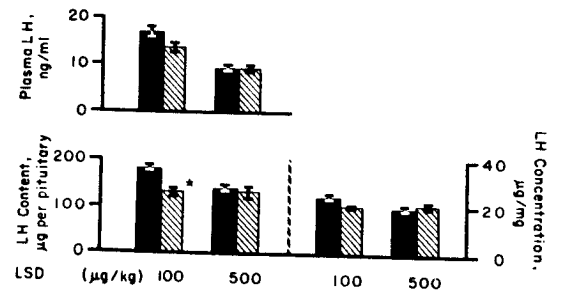

FIG. 2. Effect of chronic intraperitoneal injections of LSD on plasma and pituitary levels of LH. ■, vehicle; ▩, LSD; *, $P < 0.001$.

of biogenic amines are shown in Table 2. The low dosage significantly reduced NA and significantly increased DA, while the only effect of the high dosage was a significant reduction of 5-HIAA levels.

Discussion

The chronic administration of LSD[2] at two dose levels to prepubertal male rats significantly inhibited body growth. This inhibitory effect does not seem to be mediated through decreased food intake, since the latter was not modified by the low dosage of LSD and was only slightly reduced by the high dosage. The observed decrease in plasma or pituitary levels of GH suggests, on the contrary, a more specific mechanism of action, namely, inhibition of the synthesis and/or release of GH. In contrast to what has been observed with Δ^9-THC, the active principle of marihuana (Collu *et al.* 1975), LSD exerted no significant effects on gonadotropin levels. Although some reproductive organs had smaller absolute weights in the high dosage experimental group, when expressed as percentage of body weight they were not significantly different from those of controls. This indicates that the effect was probably due to the inhibitory action of LSD on total body growth. Several reports have appeared on the effect of LSD on the biogenic amines of the central nervous system (Leonard

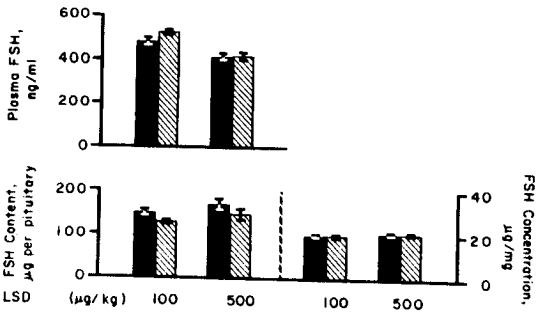

FIG. 3. Effect of chronic intraperitoneal injections of LSD on plasma and pituitary levels of FSH. ■, vehicle; ▩, LSD.

1972; Leonard and Tonge 1969; Peters 1974). These effects appear to be dose and time dependent, and the present results, which show a decrease in NA and an increase in DA at low dosage and a decrease in 5-HIAA at high dosage, are in general accord with the literature. These results suggest that LSD may exert its actions by decreasing the turnover of 5-HT and modifying the brain levels of catecholamines. It should be pointed out that although LSD is excreted rapidly in rodents, its repeated administration can induce changes in monoamine turnover that persist for a longer period of time (Peters 1974). No explanation can be offered for the differences observed between the effects on biogenic amines obtained with a relatively low dose and those obtained with a high dose; however, this has already been reported (Peters 1974). Interestingly enough, different effects on plasma and pituitary GH levels were also observed with the two doses, as previously noted. Since we have reported that the secretion of GH in the rat is under a dual, mutually antagonistic control system, serotoninergic stimulatory, and dopaminergic inhibitory (Collu *et al.* 1972), the inhibitory effect of LSD on growth and GH could thus be due to a modification of this system. A recent paper by us (Collu *et al.* 1974) shows that selective degeneration of brain serotoninergic nerve terminals by 5,6-dihydroxytryptamine in prepubertal male rats also induced growth retardation and decreased pituitary GH content. However, further studies are needed to verify whether LSD-induced changes in biogenic amine metabolism occur in brain regions such as hypothalamus, amygdala, and hippocampus, which are known to be able to control the secretion of GH. Finally, it is well known that plasma levels of GH are readily depressed by stress in the rat (Schalch and Reichlin 1966; Collu *et al.* 1973*c*); it is therefore possible that the effects of LSD are due to non-specific chronic stress. Although plasma levels of corticosterone have not been measured, the unmodified weights of the adrenal glands do not support this hypothesis.

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ANSELL, G. B., and BEESON, M. F. 1967. A rapid and sensitive procedure for the combined assay of noradrenaline, dopamine, and serotonin in a single brain sample. Anal. Biochem. **23**, 196–206.

COLLU, R., FRASCHINI, F., and MARTINI, L. 1973*a*. Role of indoleamines and catecholamines in the control of gonadotrophin and growth hormone secretion. *In* Progress in brain research. Vol. 39. *Edited by* E. Zimmermann, W. H. Gispen, B. H. Marks, and D. De Wied. Elsevier, Amsterdam. pp. 289–299.

COLLU, R., FRASCHINI, F., VISCONTI, P., and MARTINI, L. 1972. Adrenergic and serotoninergic control of growth hormone secretion in adult male rats. Endocrinology, **90**, 1231–1237.

COLLU, R., JEQUIER, J.-C., LETARTE, J., LEBŒUF, G., and DUCHARME, J. R. 1973*b*. Diurnal variations of plasma growth hormone and brain monoamines in adult male rats. Can. J. Physiol. Pharmacol. **51**, 890–892.

——— 1973*c*. Effect of stress and hypothalamic deafferentation on the secretion of growth hormone in the rat. Neuroendocrinology, **11**, 183–190.

——— 1974. Endocrine effects of brain serotonin depletion by 5,6-dihydroxytryptamine in prepubertal male rats. Neuroendocrinology, **14**, 139–150.

COLLU, R., LETARTE, J., LEBŒUF, G., and DUCHARME, J. R. 1975. Endocrine effects of chronic administration of psychoactive drugs to prepubertal male rats. I: Δ^9-tetrahydrocannabinol. Life Sci. **16**, 533–542.

LEONARD, B. E. 1972. Hallucinogenic drugs and central transmitter substances. Int. J. Neurosci. **4**, 35–43.

LEONARD, B. E., and TONGE, S. R. 1969. The effects of some hallucinogenic drugs upon the metabolism of noradrenaline. Life Sci. **8**, (Part I), 815–825.

MILLER, F. P., COX, R. H., JR., SNODGRASS, W. R., and MAICKEL, R. P. 1970. Comparative effects of *p*-chlorophenylalanine, *p*-chloroamphetamine and *p*-chloro-*N*-methylamphetamine on rat brain norepinephrine, serotonin and 5-hydroxyindole-3-acetic acid. Biochem. Pharmacol. **19**, 435–442.

NISWENDER, G. D., MIDGLEY, A. R., JR., MONROE, S. E., and REICHERT, L. E. 1968. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-^{131}I. Proc. Soc. Exp. Biol. Med. **128**, 807–811.

PARLOW, A. F., DAANE, T. A., and SCHALLY, A. W. 1969. Quantitative, differential measurement of rat serum FSH, LH and hypothalamic FSH-RH and LH-RH with specific radioimmunoassays. Progress of the 51st Meeting of the Endocrine Society. Vol. 88. p. 83. (Abstr.).

PETERS, D. A. V. 1974. Comparison of the chronic and acute effects of D-lysergic acid diethylamide (LSD) treatment on rat brain serotonin and norepinephrine. Biochem. Pharmacol. **23**, 231–237.

SCHALCH, D. S., and REICHLIN, S. 1966. Plasma growth hormone concentration in the rat determined by radioimmunoassay. Influence of sex, pregnancy, lactation, anesthesia, hypophysectomy and extrasellar pituitary transplants. Endocrinology, **79**, 275–280.