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COMPARISON OF THE CHRONIC AND ACUTE EFFECTS OF D-LYSERGIC ACID DIETHYLAMIDE (LSD) TREATMENT ON RAT BRAIN SEROTONIN AND NOREPINEPHRINE

DAVID A. V. PETERS

Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Canada

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Abstract—The effects of repeated administration of small doses of D-lysergic acid diethylamide (LSD) on brainstem norepinephrine (NE), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tyrosine, tryptophan, tyrosine hydroxylase and tryptophan hydroxylase have been studied in male Sprague–Dawley rats. A daily dose of 20 μg/kg given for 14 days resulted in a significantly lowered NE level and tyrosine hydroxylase activity measured 24 hr after the last injection. Treatment with 100 μg/kg/day for the same period of time produced similar changes together with a significantly lower brainstem tyrosine content. Evidence was obtained that the NE turnover in rat brainstem was significantly increased at the higher dose level only. The lower dose of 20 μg/kg/day also resulted in an elevated 5-HT and lowered 5-HIAA content. However, when the dose was increased to 100 μg/kg/day the 5-HT content was unchanged and the 5-HIAA content increased. The 5-HT turnover was reduced when the rats were treated with 20 μg/kg/day but significantly elevated when the 100-μg dose was used.

NUMEROUS reports have described adverse reactions after repeated use of D-lysergic acid diethylamide (LSD) by human subjects.[1–5] Many users describe "flashbacks" or occasional transient returns to the hallucinatory state long after the immediate effects of the drug have worn off.[4,5] Since LSD is rapidly excreted,[6] these effects may represent a persistent metabolic change in brain.

A single administration of LSD to experimental animals produces a number of physiological and behavioral effects.[7–15] It has been known for several years that these effects are paralleled by small but statistically significant changes in the levels of norepinephrine (NE), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in brain,[16–18] and several investigators have suggested possible relationships between NE and 5-HT and the physiological and behavioral effects of the drug.[19–23]

LSD is believed to block release of 5-HT from nerve endings[24–26] which may be the origin of the observed changes in 5-HT and 5-HIAA levels[16,17] after administration of a single dose of the drug to experimental animals. Using microiontophoretic techniques, LSD has been shown to block the firing of serotonergic neurons in the rat midbrain raphe[27] and to inhibit the 5-HT-induced excitation of neurons from several brain areas.[23,28] LSD also stimulates certain serotonergic neurons in the CNS.[29]

Reserpine potentiates the psychotomimetic effects of LSD in man[30] and the behavioral changes in rats[14] which may be consistent with an interaction of LSD with 5-HT in the mechanisms involved in the formation of the psychotomimetic

effects. However, reserpine also depletes NE, and it is significant that α-methyl-*p*-tyrosine, a catecholamine-depleting agent, abolished the LSD-induced excitation in rabbits.[13] Leonard and Tonge[18] reported that LSD caused a significant decrease in rat brain NE and slightly elevated dopamine content, although in this case α-methyl-*p*-tyrosine had no effect on the gross behavioral changes produced by LSD.[31] LSD has also been reported to produce a moderate increase in the catecholamine fluorescence in the septal region of rat brain.[32] Dixon[33] investigated the effect of a very large dose of LSD on rats and suggested that the distinctive pattern of arousal which resulted was mediated through the catecholamines and not 5-HT.

Little is known of the effects of repeated administration of LSD to experimental animals despite the fact that abuse of the drug by human subjects frequently involves chronic use over a long period of time. Since there is considerable evidence to link at least some of the acute effects of the drug to an interaction between LSD and noradrenergic and serotonergic systems in brain, we decided to start a study of the chronic effects of LSD in the rat with an investigation of its effect on 5-HT and NE synthesis, utilization and breakdown. We now report that chronic LSD treatment to rats produces a persistent change in the levels and turnover rates of both 5-HT and NE in brain.

MATERIALS AND METHODS

For the acute study, male Sprague–Dawley rats (160–180 g) were injected by the intraperitoneal route with 500 μg/kg of LSD dissolved in saline (0·2 ml/100 g). Control animals received saline alone. The rats were killed by decapitation either 0·5, 4 or 24 hr after the LSD injection. In a further group of experiments, the same dose of LSD was divided into five equal portions and given at half-hourly intervals, the rats being killed 0·5 hr after the final injection. For the chronic study the rats were treated similarly except that the injections were given daily and the rats sacrificed 24 hr after the final injection. In both cases the brains were removed and homogenized in either 10 ml of acidified butanol (0·2 ml of 10 N HCl in 200 ml *n*-butanol) or in 4–6 vol. of ice-cold 0·25 M sucrose. The butanol homogenates were used for the estimation of 5-HT, 5-HIAA and NE by the methods described by Curzon and Green[34] and Maickel *et al.*[35] An aliquot of the sucrose homogenate was used to measure tryptophan-5-hydroxylase[36] and tyrosine hydroxylase[37] activities. A 1-ml aliquot of the remaining sucrose homogenate was diluted with 5 ml of 6% trichloroacetic acid and centrifuged. The supernatant was used to assay tryptophan[38] and tyrosine.[39]

Some of the rats were treated with pargyline (100 mg/kg) injected intraperitoneally 1 hr before sacrifice. Measurement of the increase in brain 5-HT and NE levels produced by this inhibitor of monamine oxidase allowed an estimate of the 5-HT and NE turnover to be made.[40]

RESULTS

The results of various LSD treatments on rat brain 5-HT, 5-HIAA, tryptophan and tryptophan-5-hydroxylase are given in Table 1. A single 500 μg/kg injection of LSD resulted in a significant increase in brain 5-HT content (12 per cent) and a concomitant decrease in brain 5-HIAA (14 per cent). The tryptophan concentration was slightly but not significantly ($P = 0{\cdot}1$) decreased while the tryptophan hydroxylase activity was unchanged. Slightly greater changes were obtained when the same dose

TABLE 1. EFFECT OF LSD TREATMENT ON RAT BRAINSTEM 5-HT, 5-HIAA, TRYPTOPHAN AND TRYPTOPHAN HYDROXYLASE*

LSD treatment	5-HT		5-HIAA		Tryptophan		Tryptophan-5-hydroxylase	
	(μg/g)	(%)	(μg/g)	(%)	μg/g)	(%)	(nmoles/g/hr)	(%)
Acute								
Control	0·94 ± 0·02	100	0·56 ± 0·03	100	4·98 ± 0·16	100	8·92 ± 0·35	100
500 μg/kg	1·10 ± 0·07	112	0·48 ± 0·07	86	4·82 ± 0·20	97	8·85 ± 0·41	99
5 × 100 μg/kg	1·12 ± 0·07*	114	0·46 ± 0·03†	82	4·77 ± 0·21	96	8·76 ± 0·48	98
Chronic (14 days)								
Control	0·96 ± 0·01	100	0·57 ± 0·02	100	5·27 ± 0·14	100	8·78 ± 0·35	100
20 μg/kg/day	1·05 ± 0·06	110	0·43 ± 0·04†	75	4·96 ± 0·26	94	7·89 ± 0·45	90
100 μg/kg/day	0·89 ± 0·05	94	0·77 ± 0·04†	135	6·03 ± 0·22†	114	8·70 ± 0·70	99

* Results are given as mean ± S. E. M. for a minimum of five animals in each group.
† Denotes values which are significantly different from control values at $P < 0·05$.

of LSD was given in five equal portions at half-hourly intervals. In both experiments, the animals were killed 30 min after the final LSD injection. Alternatively, when the rats were killed 4 or 24 hr after LSD treatment, there were no detectable differences between experimental and control animals.

Similar results were obtained when a much smaller dose of LSD (20 μg/kg) was given daily for 14 days. However, whereas the changes following an acute dose of LSD were found only a short time after the treatment, following chronic administration of the drug, the changes were significant 24 hr after the final injection.

A higher dose level of LSD (100 μg/kg/day) for the same period of time (14 days) resulted in a different pattern of changes. The 5-HT content was not significantly different from the control level but the 5-HIAA level was markedly increased, an indication of an increased 5-HT turnover rather than the decrease found before. Additional information on the effect of LSD on 5-HT level after inhibition of breakdown gives an indication of relative turnover rates.[40] The results are shown in Table 2. In control animals, pargyline caused approx. 100 per cent increase in brain serotonin content. However, after a single LSD injection (500 μg/kg) pargyline pretreatment increased the serotonin content by only 77 per cent suggesting a decreased serotonin turnover, although the difference was not statistically significant ($P = 0{\cdot}08$). A similar result was obtained after the chronic treatment with the lower dose schedule (20 μg/kg/day). The higher dose schedule resulted in a markedly greater accumulation of serotonin than in control animals (137 per cent) which is consistent with an increased turnover. Both methods of detecting changed turnover rates were, therefore, consistent with an increased serotonin turnover after 2 weeks of treatment with 100 μg/kg/day of LSD.

TABLE 2. EFFECT OF LSD AND PARGYLINE ON RAT BRAINSTEM 5-HT*

LSD treatment	5-HT (μg/g)		
	Without pargyline	With pargyline	Change (%)
Acute			
Control	0·85 ± 0·04	1·71 ± 0·03	+101
500 μg/kg	0·99 ± 0·06	1·75 ± 0·11	+77
Chronic (14 days)			
Control	0·86 ± 0·03	1·69 ± 0·02	+96
20 μg/kg/day	0·95 ± 0·06	1·53 ± 0·04	+61*
100 μg/kg/day	0·80 ± 0·05	1·90 ± 0·05	+137†

* Results are given as mean ± S. E. M. for a minimum of five animals in each group.

† Denotes that the increase in 5-HT produced by pargyline treatment is significantly greater than in control animals.

The effects of LSD treatment on rat brain NE, tyrosine and tyrosine hydroxylase are shown in Table 3. Neither a single large dose of LSD (500 μg/kg) nor the same dose given in five equal portions at half-hourly intervals produced any significant change in brain NE, tyrosine or tyrosine hydroxylase. However, treatment with both high and

low chronic dose schedules resulted in a significantly lower NE level and tyrosine hydroxylase activity. The brain tyrosine content was lower than control values in both groups but the decrease was statistically significant only after the higher chronic dose.

TABLE 3. EFFECT OF LSD TREATMENT ON RAT BRAINSTEM NE, TYROSINE AND TYROSINE HYDROXYLASE*

	NE		Tyrosine		Tyrosine hydroxylase	
LSD treatment	(μg/g)	(%)	(μg/g)	(%)	(nmoles/g/hr)	(%)
Acute						
Control	0·50 ± 0·04	100	20·9 ± 0·6	100	16·9 ± 0·5	100
500 μg/kg	0·49 ± 0·06	98	21·0 ± 0·7	100	16·6 ± 0·5	98
5 × 100 μg/kg	0·49 ± 0·01	98	20·5 ± 1·4	98	16·9 ± 0·6	100
Chronic (14 days)						
Control	0·45 ± 0·02	100	22·2 ± 0·5	100	16·5 ± 0·3	100
20 μg/kg/day	0·37 ± 0·02†	81	20·9 ± 0·9	94	14·6 ± 0·5†	88
100 μg/kg/day	0·38 ± 0·01†	84	18·4 ± 0·7†	83	13·5 ± 0·5†	82

* Results are given as mean ± S. E. M. for a minimum of five animals in each group.
† Denotes values which are significantly different from the control values at $P < 0·05$.

The effects of LSD treatment on brain NE turnover are summarized in Table 4. A single injection of 500 μg/kg produced evidence of a slight and not statistically significant decrease in NE turnover. Treatment with pargyline produced a greater accumulation of NE in the brainstem of rats previously exposed to 100 μg/kg/day of LSD for 14 days than in control animals.

TABLE 4. EFFECT OF LSD AND PARGYLINE ON RAT BRAINSTEM NE*

	NE (μg/g)		
LSD treatment	Without pargyline	With pargyline	Change (%)
Acute			
Control	0·53 ± 0·06	0·82 ± 0·05	+55
500 μg/kg	0·49 ± 0·06	0·71 ± 0·09	+45
Chronic (14 days)			
Control	0·45 ± 0·01	0·72 ± 0·04	+59
20 μg/kg/day	0·37 ± 0·02†	0·57 ± 0·03	+56
100 μg/kg/day	0·38 ± 0·01†	0·76 ± 0·04	+103‡

* Results are given as mean ± S. E. M. for a minimum of five animals in each group.
† Denotes values which are significantly lower than control values at $P < 0·05$.
‡ Denotes that the increase in NE produced by pargyline treatment is significantly greater than in control animals.

DISCUSSION

A single large dose of LSD (500 μg/kg) produced similar changes in brain 5-HT metabolism to those reported by others,[16–18] notably increased 5-HT content and reduced 5-HIAA level. The 5-HT turnover appeared to be reduced, although in this experiment the difference was not quite statistically significant ($P = 0{\cdot}08$). Lin *et al.*[41] report a significant increase, although in this case a higher dose of LSD (1 mg/kg) was used. However, we found no significant changes in NE content or turnover, although Leonard and Tonge[18] reported a short-lasting decrease in NE level when using a smaller dose of LSD (200 μg/kg). At present, the reason for the discrepancy between our result and that of Leonard and Tonge is not clear. However, it is significant that Freedman[17] reported a smaller decrease in rat brain NE than that described by Leonard and Tonge even though they used a much higher dose of LSD (1300 μg/kg compared with 200 μg/kg). It is possible that rat strain differences may be responsible for these inconsistencies. It is also interesting that de la Torre[32] noted a moderate increase in catecholamine fluorescence, probably due to NE, in the septal region of rat brain after LSD treatment.

LSD appears to be rapidly excreted in rodents.[6] In order to produce conditions more clearly resembling those found in man, we decided to investigate the effect of prolonging the time the LSD was in the rat tissues by administering the same dose of drug in five equal portions at half-hourly intervals. It was found that the effects on serotonin metabolism were similar to those found after a single dose but were generally enhanced. As with a single acute dose there were no significant changes in NE, tyrosine or tyrosine hydroxylase.

The increased serotonin turnover after 14 days of the 100 μg/kg/day dose schedule is in agreement with the results of Diaz and Huttunen[42] who reported a significantly increased conversion of H^3-tryptophan into serotonin after treatment of rats for 1 month with 20 μg/kg/day of LSD. In our study there were slight reductions in the activities of tryptophan-5-hydroxylase and tyrosine hydroxylase, the enzymes involved in the rate determining steps in the conversion of tryptophan to 5-HT[43] and tyrosine to the catecholamines.[44] It has been suggested that in rat brain tryptophan-5-hydroxylase is probably not saturated under normal conditions, and the rate of 5-HT synthesis is partially dependent on the brain tryptophan concentration.[36,43] Since in both this study and the work of Diaz and Huttunen[42] the brain tryptophan content was increased, it is possible that the elevated 5-HT turnover was due to an increased availability of substrate.

When the LSD dose was reduced to 20 μg/kg/day for the same period of time the results were markedly different. Evidence of a significantly reduced 5-HT turnover without change in NE turnover was found. These data and those of Diaz and Huttunen[42] would suggest that chronic LSD treatment can produce two possible effects on rat brain 5-HT and NE. First, a low daily dose of LSD (20 μg/kg/day) for a short period of time (14 days) produces results which are similar to those obtained after a single acute dose except that the changes persist for a longer period of time. Second, if either the dose level or the length of the experiment is increased (to 100 μg/kg/day or to 1 month), the effect on 5-HT and NE turnover is distinctly different from that following a single acute injection, both being significantly increased.

The changed 5-HT and NE turnover was obtained 24 hr after the last injection. By comparison, a far larger single injection of LSD produced no significant changes

even 4 hr after the treatment. No conclusion can be made at this time as to the relationship between these prolonged changes in 5-HT and NE metabolism and the persistent effects of LSD in man. However, it is highly significant that it is possible to demonstrate persistent biochemical changes in 5-HT and catecholamine metabolism in brain at a period when in all probability very little LSD remains in the tissues.

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