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PLASMA CORTICOIDS AND BRAIN TRYPTOPHAN AFTER ACUTE AND TOLERANCE DOSAGE OF LSD

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SUMMARY: The effect of acute and tolerance dosage schedules of LSD on plasma corticoids and brain tryptophan in normal, adrenalectomized and hypophysectomized rats was examined. Plasma corticoids increased 20-fold after one dose of LSD and the time course of this effect paralleled the increase in brain tryptophan. Both effects of LSD were abolished in adrenalectomized animals; after hypophysectomy the drug-induced tryptophan increase did occur but only after a significant delay. After multiple daily injections of LSD there was a significant degree of tolerance both to the plasma corticoid and brain tryptophan response.

Neurochemical changes in indoleamines following an acute dose of LSD and psychoactive congeners include an increase of brain 5-hydroxytryptamine (5-HT) and a decrease in 5-hydroxyindoleacetic acid (5-HIAA)(1). The decrease in 5-HIAA indicates a slowed turnover of 5-HT (2-5) and there is an inhibition of 5-HT synthesis from labelled tryptophan (6). Oddly, however, brain tryptophan increases after an acute dose of LSD. This is paradoxical since inhibition of 5-HT synthesis is generally accompanied by reduced levels of brain tryptophan whereas increased levels of tryptophan are associated with enhanced synthesis of 5-HT (for review)(7). The increase in brain tryptophan was not associated, in the present experiments, with an elevation of free plasma tryptophan, even though increases in free plasma tryptophan are generally linked with augmented levels of brain tryptophan. The increase in brain tryptophan is diminished with a tolerance dosage schedule of LSD but the turnover of 5-HT remains reduced although time parameters are shifted (8). This suggests some independence of the tryptophan effects from those on 5-HT turnover.

Since hydrocortisone injections may elevate rat brain tryptophan levels (9) and increase tryptophan uptake into nerve-endings (10), we studied the effect of LSD on both plasma corticoids and brain tryptophan to see if the paradoxical tryptophan effect might be contingent on steroid response. In man, plasma cortisol had been found to be elevated during the LSD-induced period of emotional excitement (11) and in rat LSD reportedly induced pronounced increases in free 17-OH corticosteroid levels and moderate increases in the 17-ketosteroid levels (12-13).

Material and Methods

Animals. Male Sprague-Dawley rats (Madison, Wis.) 250-300 g in weight, were used in all experiments. Bilaterally adrenalectomized, hypophysectomized and sham-operated rats (150-200 g) were purchased from the supplier immediately after the operation. They were housed six in a cage at constant temperature and

relative humidity under a 12 hr light/12 hr dark cycle. Animals had ad libitum access to Purina rat chow and water. Operated animals were used 5-8 days following the operation. All animals were sacrificed between 10 and 12 a.m.

Plasma Corticoids. Rat plasma corticoids were determined by competitive protein-binding assay technique as described by Nugent and Mayers (14).

Brain 5-HT and 5-HIAA. Serotonin was assayed by the method of Andén and Magnusson (15) and 5-HIAA was determined by the method of Jonsson and Lewander (16).

Tryptophan. Brain tryptophan was assayed by the method of Denckla and Dewey (17). Plasma tryptophan was separated into free and bound as described by Knott and Curzon (18) and further determined by the method of Denckla and Dewey.

LSD. LSD bitartrate was injected i.p. in a dose of 520 µg/kg dissolved in saline (2.5 ml/kg). Control animals received equivalent volumes of saline.

Results and Discussion

Following a single i.p. injection of LSD (520 µg/kg), the parallel increase over time of both plasma corticoids and brain tryptophan was evident. Maximal increase was reached at 60 min and there was a decline thereafter (Table 1).

TABLE 1

Effect of LSD on Plasma Corticoids and Brain Tryptophan in the Rat[a]

Treatment	Plasma Corticoids (µg/100 ml)	% increase[b]	Brain Tryptophan (µg/g)	% increase[b]
Saline	2.6 ± 0.3	----	4.4 ± 0.1	--
LSD 30 min	55.1 ± 1.9	2019	5.5 ± 0.2	25
LSD 60 min	57.7 ± 2.1	2119	6.0 ± 0.3	36
LSD 90 min	21.2 ± 3.1	715	5.1 ± 0.1	16

[a]Plasma corticoids and brain tryptophan were assayed in the same animals following a single i.p. injection of LSD (520 µg/kg) in a volume of 2.5 ml/kg. There were 10-12 animals in each group at each time point. Values are the mean ± S.E.

[b]Significant changes ($p<0.005$, two-tailed Student's t test) with respect to saline.

TABLE 2

Effect of LSD on Total and Free Plasma Tryptophan in the Rat[a]

Treatment	Total Plasma Tryptophan (µg/ml)	Free Plasma Tryptophan (µg/ml)
Saline	20.9 ± 0.6 (16)	3.4 ± 0.14 (11)
LSD 60 min	19.8 ± 0.8 (14)	3.0 ± 0.16 (11)

[a]Total and free plasma tryptophan were assayed following a single i.p. injection of LSD (520 µg/kg) in a volume of 2.5 ml/kg. Values are the mean ± S.E for the number of animals given in parentheses.

TABLE 3

Effect of LSD on Brain 5-HT, 5-HIAA, Tryptophan and on Plasma Corticoids in Adrenalectomized and Hypophysectomized Rats[a]

Treatment	Adrenalectomized				Hypophysectomized			
	5-HT (ng/g)	5-HIAA (ng/g)	Tryptophan (µg/g)	Corticoids (µg/100 ml)	5-HT (ng/g)	5-HIAA (ng/g)	Tryptophan (µg/g)	Corticoids (µg/100 ml)
Saline	498 ± 5	300 ± 26	4.4 ± 0.1	N.M.	502 ± 10	303 ± 13	4.4 ± 0.1	N.M.
LSD 30 min	N.D.	N.D.	4.4 ± 0.2	N.M.	513 ± 16	180 ± 21[b]	4.7 ± 0.2	N.M.
LSD 60 min	623 ± 6[b]	183 ± 13[b]	4.3 ± 0.3	N.M.	603 ± 22[b]	208 ± 18[b]	4.9 ± 0.2	N.M.
LSD 90 min	N.D.	N.D.	4.6 ± 0.2	N.M.	590 ± 12[b]	198 ± 23[b]	5.5 ± 0.2[b]	N.M.

[a] Plasma corticoids and brain 5-HT, 5-HIAA and tryptophan were assayed in the same animals following a single i.p. injection of LSD (520 µg/kg) in a volume of 2.5 ml/kg. There were 8 animals in each group at each time point. Values are the mean ± S.E.

N.D. = not determined; N.M. = not measurable

[b] $p<0.05$ (two-tailed Student's *t* test)

There was no statistically significant effect of LSD on either total or free plasma tryptophan (Table 2). There was a trend toward a decrease in both total and free plasma tryptophan and further experiments are now in progress.

Following adrenalectomy, plasma corticoid levels were not measurable either in control or in LSD-treated animals nor was there a tryptophan response to LSD over a 90 minute period. In hypophysectomized animals receiving saline or LSD plasma corticoids were not measurable, but brain tryptophan showed a significant increase after LSD at 90 minutes rather than at 60 minutes (Table 3). The delayed tryptophan response to LSD in hypophysectomized animals is unexplained. The effects of hypophysectomy alone upon factors regulating plasma and brain tryptophan would be of interest. For example, indices of increased sympathetic activity, measures of plasma unesterified fatty acids, glucose and amino acid concentrations (19) before and after LSD in hypophysectomized animals might elucidate this finding.

LSD increased brain 5-HT and decreased 5-HIAA in the adrenalectomized and hypophysectomized rats following a dose of 520 µg/kg. While adrenalectomy affects the conversion index of tryptophan and the activity of tryptophan hydroxylase in brain, the procedure does not alter steady-state levels of 5-HT (20) and indeed neither the magnitude nor the time course of the LSD effect on 5-HT and 5-HIAA were changed by either of the operations. The effect of LSD in slowing down 5-HT turnover has been attributed to several mechanisms including an inhibition of both raphe firing (21) and 5-HT release (22) and enhanced vesicular binding (2,23). The findings in the adrenalectomized animals indicate that the slowed-down turnover is, in any event, not related to either the increase of tryptophan or the corticoids.

Multiple dosages of LSD diminished both the magnitude and duration of the tryptophan and corticoid response following a single dose (Fig. 1). After 3, 5 or 7 daily LSD injections there was a parallel decrement both in plasma corticoids and brain tryptophan at all time points after the last LSD injection. The magnitude of the decrement paralleled the number of injections and was greatest after the seventh dose. At that time both tryptophan and the corticoids peaked at 30 (rather than at 60) min and levels declined rapidly thereafter; at 90 min tryptophan and the corticoids were at control levels. A diminished duration and/or magnitude of effect characterizes tolerance both to biochemical and behavioral effects in accord with previous reports from our laboratory (8,24,25). These findings further suggest a close link between steroid response and brain tryptophan. The tolerance effect on corticoids is striking and thus may be of some practical value in psychopharmacological studies where elaborate behavioral measures to track tolerance are not feasible.

It is not clear how LSD causes ACTH release. LSD can exert both agonistic and antagonistic actions on central 5-HT receptors and, in turn, 5-HT levels have been shown to covary diurnally with steroids (26); similarly, the circadian rise of plasma corticosteroids was abolished by drugs which drastically alter serotonin levels (27). Indeed, serotonergic mechanisms have been implicated in the regulation of the hypothalamic-pituitary-adrenal system (28-31). Whether the initial corticoid response and tolerance to it reported here are related to the effects of LSD on serotonin or norepinephrine (32,33) remains to be established.

These findings do indicate that the LSD-induced slow-down of 5-HT turnover is not associated with the effect on brain tryptophan. Nor does the tryptophan increase after a single dose appear to be a compensatory phenomenon for the reduced synthesis and turnover of 5-HT since there was no tryptophan increase after adrenalectomy although there was an increase in 5-HT and a decrease in 5-HIAA.

Thus, LSD presumably increases brain tryptophan via adrenal steroids. The precise mechanism by which steroids enhance levels of brain tryptophan, such as binding to a common carrier, requires elucidation. While these experiments clarify a paradoxical phenomenon in the mode of action of LSD, they do highlight a more general question of the adaptive function of the link of steroids to amino acid levels and transport.

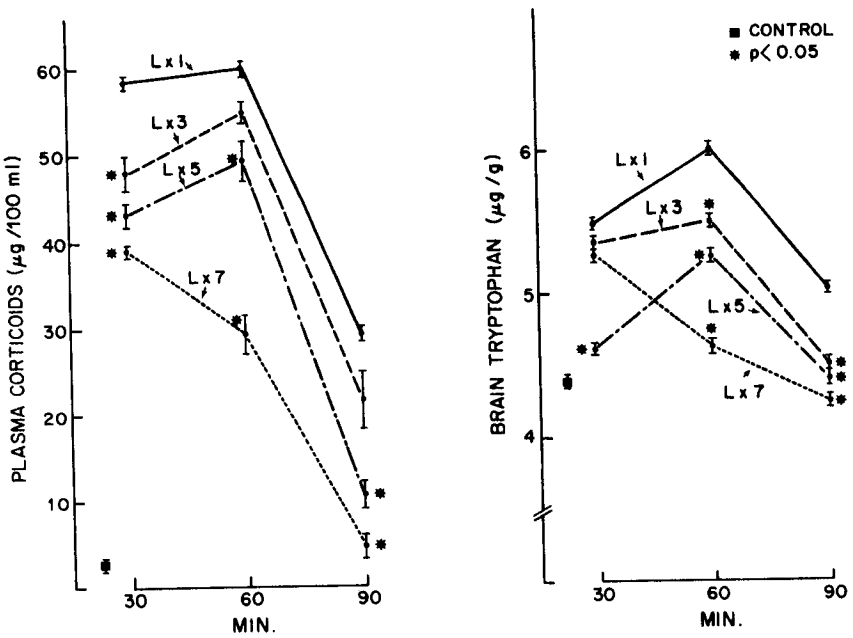

FIG. 1

Effect of single (Lx1) and multiple (Lx3, Lx5, Lx7) injections of LSD (520 µg/kg, i.p.) on plasma corticoids and brain tryptophan. Following a single dose all changes are significantly different from control ($p<0.05$). Asterisks indicate that a given value is significantly different when compared to the corresponding value after a single dose. There were 8 animals in each group at each time point. Values are the mean ± S.E. and statistical significance was determined by the two-tailed Student's t test. Two-way analysis of variance gave the following values for plasma corticoids: F_A 165.14; F_B 122.14; F_{AxB} 81.35. The corresponding values for brain tryptophan are: F_A 25.88; F_B 23.66; F_{AxB} 5.11. All F-ratios in both measures are statistically significant at the $p<0.001$ level.

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