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PERSISTENT EFFECTS OF CHRONIC ADMINISTRATION OF LSD ON INTRACELLULAR SEROTONIN CONTENT IN RAT MIDBRAIN

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Summary—The effects of acute and chronic administration of LSD on the intracellular serotonin content of raphe neurons in rat midbrain were compared using a cytofluorimetric measure of serotonin based on fluorescence fading. Consistent with previous reports, acute treatment with LSD preferentially increased intracellular serotonin levels in both the dorsal and median raphe nuclei. Similar increases in serotonin were observed following 14 daily injections of LSD and 24 hr after the last of 13 daily injections. No significant differences were found between the effects of acute and chronic LSD treatments on any of the cytofluorimetric measures of serotonin. Together with the results from previous electrophysiological and behavioral studies, these findings indicate that little or no tolerance occurs to the effects of LSD on serotonergic neurons.

The behavioral effects of Lysergic Acid Diethylamide (LSD) have often been suggested to be related to its marked alterations of serotonergic neurotransmission in brain. Even small doses of LSD or related hallucinogens have powerful inhibitory effects on the rate of firing of the serotonergic cells in the midbrain raphe nuclei. Various hallucinogens also reduce the turnover rate of brain serotonin (5-HT) (Brawley and Duffield, 1972) and preferentially increase intracellular levels of 5-HT within midbrain raphe neurons (Geyer, Dawsey and Mandell, 1978a; Geyer, 1980). Behaviorally, hallucinogens have been studied in a variety of animal paradigms. One behavior that appears to be modulated by brain 5-HT and is affected by hallucinogens is the rat startle response to either acoustic or tactile stimuli (Davis, 1980; Geyer, Puerto, Menkes, Segal and Mandell, 1976; Geyer, Peterson, Rose, Horwitt, Light, Adams, Zook, Hawkins and Mandell, 1978b). It has been suggested that the increase in startle response following hallucinogens is related to the inhibition of firing of raphe neurons produced by these drugs (Davis, 1980).

Since the psychological effects of hallucinogens seem to exhibit rapid tolerance in humans (Balestrieri and Fontanari, 1959; Bridger, Mandell and Stoff, 1973), animal studies have attempted to extend the putative relationship between the behavioral effect of LSD and its electrophysiological or neurochemical effects on 5-HT systems by assessing the development of tolerance on these parameters. For example, Jacobs and co-workers have reported that little or no tolerance occurred to the LSD-induced inhibition of raphe cell firing following various chronic regimens in rats or cats (Trulson, Ross and Jacobs, 1977). However, the characteristic behavioral effects of LSD in cats do exhibit vary rapid tolerance (Trulson and Jacobs, 1977). In rats, some behavioral effects of hallucinogens show tolerance while other effects may be potentiated, even in the same animals (Braff and Geyer, 1980, Bridger *et al.*, 1973). For example, it has been reported that while the impaired habituation of tactile startle response produced acutely by LSD (Geyer *et al.*, 1978b) was not seen after 8 daily injections of the drug, the LSD-induced increase in the response to the first stimulus was potentiated by the chronic treatment (Braff and Geyer, 1980).

In a different context, a new cytofluorimetric method has been used to demonstrate that LSD and other hallucinogens preferentially increase 5-HT levels inside the serotonergic neurons of the midbrain raphe nuclei (Geyer *et al.*, 1978a; Geyer, 1980). This method is based upon a measure of fluorescence fading of the fluorophore derived by condensation of 5-HT with formaldehyde and has been validated in both model droplet and *in vivo* studies (Geyer *et al.*, 1978a). In the present study, the degree to which tolerance occurs to the effect of LSD on the 5-HT content of raphe neurons following 2 weeks of daily injections has been assessed.

METHODS

Animals

Twenty-four male Sprague–Dawley rats (Hilltop Laboratories, Scottdale, PA) ranging from 200 to 250 g were divided into four groups of six. Group 1 received 14 daily intraperitoneal (i.p.) injections of saline. Group 2 received 13 daily saline injections and on day 14 received a single intraperitoneal injection of LSD. Group 3 received 13 daily LSD injections and on day 14 received an injection of saline. Group 4 received 14 daily LSD injections. The dose of LSD was 100 μg/kg and all animals were sacrificed 1 hr after the last injection on day 14.

Apparatus

The microspectrofluorimeter is described in detail elsewhere (Geyer *et al.*, 1978a). Briefly, a 1000-W Xenon lamp and grating monochromater (Schoeffel) provided 410 nm excitation light that is admitted to the specimen under computer control through an electronic shutter. A Leitz microscope and MPV system with sub-stage phase-contrast red light and a Ploem epi-illuminator allowed for alignment of the specimen in phase-contrast and the isolation of a 5 μm circular area of the cytoplasm adjacent to the nucleus for fluorescence measurement. Alternatively, the measurement aperture was aligned in the regions between cell bodies. This extraperikaryal measure has been found to response to some drugs independently of changes in the intracellular measures (Geyer *et al.*, 1978a). A small grating mono-chromater is used to select the optimal emission wave-length (512 nm) for biogenic amines to be detected by the photometer. After alignment of each cell, readings were taken automatically at 1 per sec and stored on magnetic tape for subsequent analyses. Both model droplet analyses and *in vivo* studies with pargyline have shown that the fluorescence intensity remaining after 14 sec of excitation is proportional to the concentration of catecholamine, while a fading measure—the difference in fluorescence intensities from the first to the fourteenth second of excitation—is the best predictor of 5-HT concentration and is independent of catecholamine levels. To allow reliable comparisons to be made among a large number of tissue samples, the formaldehyde histochemical method has been modified so that all samples within an experiment are batch-processed together from sacrifice until sectioning.

Microscopy

Using red-light phase-contrast, the slides containing the regions of interest were selected by reference to predetermined landmarks. In this experiment, one rostrocaudal plane through the midbrain was selected for the dorsal (B7) and median (B8) raphe measures. The B7D area was designated as the dense cell cluster immediately below the aqueduct of Sylvius; B7V is the ventromedial part of B7; and B8 is the median nucleus just below the decussation of the superior cerebellar peduncle. For each region, at least 6 intracellular and 6 extraperikaryal readings were taken from each of two sections from each animal. Sections were chosen from different although adjacent slides. Four background readings were taken from non-fluorescent cell bodies in the lateral reticular formation.

Tissue preparation

One hour after injection of LSD or saline on day 14, animals were sacrificed by decapitation and their brains were removed within 90 sec. After dissection, as described previously (Geyer *et al.*, 1978a), brain samples were frozen in propane, freeze-dried over P_2O_5 for four weeks at −60°C, treated with gaseous formaldehyde, embedded in paraffin, and sectioned at 8 μm on a rotary microtome. Sections were mounted on slides in Entellan (Merck).

Statistics

Separate analyses of variance (ANOVA) were performed for both intracellular and extraperikaryal measures from each brain region examined. The appropriate values from each animal were averaged after subtraction of the corresponding blank value so that each animal contributed only one value to each ANOVA. The Newman–Keuls test (Winer, 1971) was used to make specific comparisons between groups when the overall ANOVA revealed a significant group effect.

RESULTS

The effects of acute and chronic administration of LSD on the cytofluorimetric measures of 5-HT fluorescence are summarized in Table 1. As expected from previous studies (Geyer *et al.*, 1978a; Geyer, 1980), acute treatment with LSD preferentially increased the intracellular fading measures of 5-HT in all three regions examined. The extraperikaryal measure was affected significantly only in the ventromedial B7 area and this increase was smaller than the corresponding increase intracellularly.

Table 1. Effects of LSD on the 5-HT content of raphe neurons

	Saline	Acute LSD	Chronic LSD	Acute + chronic	F (3, 20)	$P <$
Dorsal raphe						
Dorsal part						
Intracellular	958 ± 68	1803* ± 98	1536* ± 274	1476* ± 178	4.13	0.05
Extraperikaryal	982 ± 114	1161 ± 157	1310 ± 144	1103 ± 120	1.02	NS
Ventral part						
Intracellular	1098 ± 147	1941* ± 123	2231* ± 271	1957* ± 116	7.80	0.01
Extraperikaryal	1068 ± 94	1609* ± 84	1610* ± 155	1422* ± 51	6.13	0.01
Median raphe						
Intracellular	1420 ± 158	2265* ± 150	1922* ± 212	2115* ± 92	5.37	0.01
Extraperikaryal	1215 ± 66	1441 ± 86	1379 ± 102	1578* ± 69	3.35	0.05

* $P < 0.05$.

The data are expressed as group means ± SEM ($n = 6$) for the fading measure of 5-HT after subtraction of the background readings, which ranged from 200 to 300. The numbers are in nanoamperes of photometric current.

The animals sacrificed 24 hr after the thirteenth daily LSD injection also exhibited augmented levels of intracellular 5-HT in both the dorsal and median raphe. As in the acute LSD group, the only change in extraperikaryal 5-HT was in the ventromedial B7. The animals treated chronically for 13 days with LSD and given an acute treatment 1 hr before sacrifice showed the same changes in cellular 5-HT levels seen in both the acute and chronic groups: that is, intracellular 5-HT was preferentially increased in all three regions following 2 weeks of daily LSD injections. No significant differences were found between the effects of acute and chronic LSD treatments on any of the cytofluorimetric measures of 5-HT.

DISCUSSION

The present results indicate that little or no tolerance occurred to the LSD-induced augmentation of intracellular 5-HT content in midbrain raphe neurons following 14 daily administrations. In both the dorsal and median raphe nuclei, intracellular 5-HT levels were preferentially increased in animals sacrificed either 1 or 24 hr after the chronic LSD regimen. The lack of tolerance on this cytofluorimetric measure of 5-HT function is consistent with the similar findings reported for electrophysiological measures of raphe cell firing rates following acute or chronic LSD treatments (Trulson *et al.*, 1977).

An unexpected result of the present study is the finding of elevated cellular 5-HT 24 hr after the 13th daily injection of LSD. A comparison of this group with the group sacrificed one hour after the 14th LSD injection suggests that the acute administration of LSD to a chronically treated animal produced no further increase in cellular 5-HT. The persistence of the change in cellular 5-HT for 24 hr after the last injection is an intriguing finding that requires further study. The present authors are currently examining the time-course of the effect of a single dose of LSD to determine the relevance of the chronicity of treatment for this phenomenon.

The present results are also consistent with a previous report that the effect of LSD on the rat startle response to tactile stimuli did not exhibit tolerance. While the impairment of startle habituation produced acutely by LSD was diminished by 8 daily treatments, the hyper-reactivity to the initial stimuli was potentiated by the chronic LSD regimen (Braff and Geyer, 1980). In general, a variety of studies indicate that the effects of manipulations of brain 5-HT on tactile startle response can be best characterized as alterations in behavioral reactivity rather than changes in habituation (Geyer, Warbritton, Menkes, Zook and Mandell, 1975; Geyer *et al.*, 1976). Thus, reactivity to tactile stimuli appears to be modulated by brain 5-HT systems and potentiated by chronic LSD. Both the present finding of a maintained effect of LSD following chronic treatment on the cytofluorimetric measure of 5-HT function and the previous report that no tolerance occurred to the LSD-induced inhibition of raphe cell firing (Trulson *et al.*, 1977) are consistent with the lack of tolerance reported for some behavioral effects of LSD. It is conceivable, therefore, that such behavioral effects are related to the LSD-induced alterations in brain serotonergic function.

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