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LACK OF TOLERANCE TO THE DEPRESSION OF RAPHE UNIT ACTIVITY BY LYSERGIC ACID DIETHYLAMIDE

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Summary—The responsiveness of serotonin-containing midbrain raphe neurones to lysergic acid diethylamide (LSD) was examined in rats made tolerant to LSD by its repeated administration. No significant changes in the dose–response relationships between raphe unit activity and LSD administration were observed following chronic administration of low (100 μg/kg per day for 4 consecutive days) or high doses (1000 μg/kg per day for 4 or 7 days) of LSD. Similarly, no significant change was observed when rats were pretreated with LSD (250 μg/kg) every 6 hr for 4 days. In addition, no significant changes in spontaneous raphe unit activity nor duration of LSD-induced depression of unit activity occurred following chronic LSD pretreatment. These data suggest that the dramatic tolerance which develops to LSD on behavioural tests is not mediated by a change in the sensitivity of serotonin-containing neurons to LSD.

One of the most impressive behavioural effects of lysergic acid diethylamide (LSD) in humans is the dramatic tolerance which develops to its repeated administration (Abramson, Jarvik, Gorin, and Hirsch, 1956; Cholden, Kurland and Savage, 1955; Isbell, Wolbach, Wikler and Miner, 1961). While several studies have investigated the parameters of tolerance to LSD in a variety of species (Appel and Freedman, 1968; Freedman, Aghajanian, Ornitz and Rosner, 1958; Freedman, Appel, Hartman and Molliver, 1964; Winter, 1971; Trulson and Jacobs, 1977), few have dealt with the physiological mechanisms mediating tolerance. It has been shown only that the concentration of LSD in the brain and plasma is unchanged during the tolerance condition (Winter, 1971; Trulson and Jacobs, 1977), ruling out an effect due to increased peripheral metabolism.

The most prominent action of LSD in the CNS is its powerful depressant action on serotonin-containing midbrain raphe neurones (Aghajanian, Foote and Sheard, 1968). Therefore, the most obvious hypothesis to explain the dramatic tolerance which develops to repeated LSD administration would be that serotonin-containing neurones may not be as responsive to LSD under the tolerance condition. This hypothesis receives support from the finding that the LSD-induced decrease in serotonin turnover following a single injection of LSD is attenuated with its repeated administration (Freedman and Boggan, 1974). In the present study this hypothesis was tested directly by examining the dose–response curve for the effects of LSD on raphe unit activity in rats that were pretreated with several injections of LSD.

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METHODS

Units in the dorsal and median raphe nuclei were studied in male Sprague–Dawley rats (220–280 g) maintained on a 12–12 light–dark schedule. The rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and glass micropipettes (tip diameter 1–2 μm), filled with 2 M NaCl saturated with fast green (impedances ranging from 4 to 6 MΩ, measured at 1 kHz), were stereotaxically lowered into the raphe nuclei. Raphe units were identified by their characteristic slow, rhythmic firing rate (Aghajanian *et al.*, 1968; Mosko and Jacobs, 1974). With the bite bar 2.4 mm below the interaural line, dorsal raphe units were found at A 0.8–1.0, L 0.0, H (−) 0.3–1.7, and median raphe units were found at H (−) 1.8–2.8. Extra-cellular unit activity was amplified and stored on magnetic tape. On-line activity was monitored on an oscilloscope and integrated over 10 sec intervals on a strip chart writer triggered by output pulses from a Schmitt trigger derived from a pulse-height discriminator. Recording sites were marked by ejecting fast green (20 μA for 10 min) and examined histologically (50 μm thick coronal sections stained with cresyl violet).

Groups of rats were pretreated with intraperitoneal injections of either LSD tartrate (100 or 1000 μg/kg per day for 4 consecutive days) or saline (1 ml/kg per day). A single intraperitoneal injection of 100 μg/kg of LSD depressed raphe unit activity for approximately 1 hr. It was not possible to determine the time course for the depression or raphe unit activity by the 1000 μg/kg dose of LSD, because it is not technically feasible to record reliably raphe unit activity for long periods of time using currently available techniques. Unit recordings were made between 16 and 24 hr after the final injection of LSD or saline. After obtaining a stable 5-min baseline of unit activity, LSD (2.5, 5, 10 or 20 μg/kg) was injected via the femoral

vein, and unit activity was monitored for several minutes. Since intravenously administered LSD has a very rapid and short-lived effect on raphe neurones (Aghajanian *et al.*, 1968), the unit activity for minutes 2 and 3 post-injection was used in the data analysis. The dose–response data were analyzed by Probit analysis (Bliss, 1952). The statistical significance between control and experimental groups was evaluated using Student's *t*-tests.

RESULTS

Chronic LSD administration produced no significant change in the spontaneous discharge rate of raphe neurones when measured 16–24 hr after the final injection of LSD (Table 1). The effect of LSD on raphe unit activity in rats pretreated with multiple LSD injections was not significantly different ($P > 0.1$) from that in control rats given saline injections (Fig. 1). When rats were pretreated with 100 μg/kg of LSD per day, the dose–response curve was virtually identical to that found in saline-treated rats: the ED_{50} values were 5.9 and 6.4 μg/kg for saline and LSD-treated rats, respectively. When the dose of LSD was increased to 1000 μg/kg per day, the dose–response curve showed a small, non-significant shift to the right; the ED_{50} value was increased to 8.2 μg/kg. In addition, several rats (n = 8) were pretreated with LSD (1000 μg/kg per day) for 7 days, but still no significant tolerance effect was apparent. Finally, since the half-life of LSD in the rat is very short (Aghajanian *et al.*, 1968), a group of rats (n = 7) received injections of LSD (250 μg/kg) every 6 hr for 4 days. This treatment also failed to produce evidence for tolerance at the raphe unit level. The duration of depression of raphe unit activity by LSD was also not significantly changed by chronic LSD pretreatment (Table 1).

Histological analysis revealed that 89 units were in the dorsal raphe nucleus and 14 in the median nucleus.

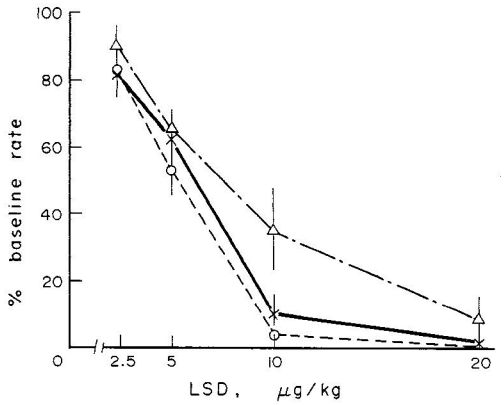

Fig. 1. Effects of LSD on raphe unit activity following chronic administration of LSD (×——×, 100 μg/kg per day for 4 days; △———△, 1000 μg/kg per day for 4 days) or saline (○----○, 1 ml/kg per day). Each point represents the mean for 6 to 10 cells, and vertical bars represent standard errors.

Table 1. Spontaneous raphe unit activity and duration of LSD-induced depression of unit activity following chronic LSD or saline administration*

Treatment	Baseline rate (Mean spikes/min ± S.E.M.)	Duration of depression (Mean min ± S.E.M.)** Dose of LSD (μg/kg)***		
		2.5	5	10
Saline (1 ml/kg per day for 4 days)	70.0 ± 7.4 (30)	2.5 ± 1.0 (6)	12.7 ± 2.1 (6)	23.0 ± 2.2 (6)
LSD (100 μg/kg per day for 4 days)	75.4 ± 12.0 (28)	3.0 ± 0.9 (6)	12.1 ± 2.3 (5)	21.2 ± 1.8 (5)
LSD (1000 μg/kg per day for 4 days)	83.8 ± 7.5 (30)	2.0 ± 0.4 (6)	11.4 ± 1.9 (7)	19.8 ± 1.7 (5)
LSD (1000 μg/kg per day for 7 days)	85.8 ± 14.4 (8)		10.8 ± 2.7 (3)	19.1 ± 2.6 (4)
LSD (250 μg/kg every 6 hr for 4 days)	76.1 ± 17.9 (7)			20.9 ± 3.1 (3)

* Rats received multiple intraperitoneal injections of LSD or saline and raphe unit activity was recorded 16–24 hr after the final injection. The n for each group is indicated in parentheses.

** The numbers represent the time required for unit activity to return to within 15% of the baseline discharge rate, since variations of this magnitude occur spontaneously.

*** Insufficient data were available for the 20 μg/kg dose, due to the difficulty in reliably recording raphe unit activity for long periods of time.

DISCUSSION

Since the discovery that LSD has a profound depressant action on serotonin-containing midbrain raphe neurones (Aghajanian *et al.*, 1968), the behavioural effects of LSD have been attributed to this action. The following model has been proposed to account for LSD-induced hallucinogenesis: because serotonin has an inhibitory post-synaptic effect, administration of LSD results in an increase in the activity of the post-synaptic target neurones owing to the removal of tonic serotonergic inhibition (Aghajanian and Haigler, 1975). There are dense serotonergic inputs to visual and limbic system structures (Fuxe, 1965; Ungerstedt, 1971), and the LSD-induced release from tonic serotonergic inhibition of these structures has been used to explain the prominent visual hallucinations and changes in affect which occur following LSD administration. If this is true, then one might expect that tolerance is mediated by a failure of LSD to disinhibit these post-synaptic target neurones following repeated administration of LSD. A corollary to this hypothesis is that LSD fails to depress raphe unit activity in the tolerance condition.

The results of the present study, however, suggest that the tolerance to LSD which occurs following its repeated administration is not mediated by failure of LSD to depress raphe unit activity in the tolerance condition. When rats were pretreated with doses of LSD known to produce tolerance using behavioural measures (Winter, 1971; Freedman *et al.*, 1964; Appel and Freedman, 1968), no significant changes were observed in the dose–response relationships between LSD administration and depression of raphe unit activity (Fig. 1). Even when the LSD pretreatment was carried out to 7 days, no significant effect was observed. Furthermore, no significant change in the responsiveness of raphe units to LSD was observed when the dose of LSD was spread out over time; i.e. administering 250 μg/kg of LSD every 6 hr for 4 days. Since the half life of LSD in rats is relatively short (20 min), this procedure maintained a high level of LSD for a prolonged period of time.

The finding that no tolerance occurs to the depression of raphe unit activity by LSD needs to be reconciled with the biochemical findings which revealed a significant tolerance effect. While it was reported that the LSD-induced decrease in serotonin turnover following a single injection of LSD was significantly attenuated with repeated LSD administration (Freedman and Boggan, 1974), the change is of a very small order of magnitude (i.e. 5–10%). It is important to note that LSD administration following chronic LSD pretreatment still results in a large change in serotonin turnover. In the present study, chronic LSD pretreatment produced a small, non-significant shift to the right in the dose–response curve relating depression of raphe unit activity to dose of LSD administered. This small shift in the dose–response curve may explain the 5–10% changes which occur in serotonin turnover measures. The important point is that the small changes in responsiveness of the serotonin system to LSD following chronic LSD pretreatment are probably not sufficient to account for the dramatic tolerance which occurs to LSD using behavioural measures.

While low doses of LSD result in an increase in the activity of post-synaptic target neurones by removing a tonic serotonergic inhibition, high doses of LSD decrease the activity of the same post-synaptic target neurones (Haigler and Aghajanian, 1974). These findings led to the hypothesis that there are two different serotonin receptors: one on raphe neurones (i.e. pre-synaptic) which is extremely sensitive to LSD, and a less sensitive one on neurones post-synaptic to raphe cells. This hypothesis receives support from studies which revealed that raphe neurones and neurones post-synaptic to them are equally sensitive to microiontophoretically applied serotonin, while raphe neurones are much more sensitive to microiontophoretically applied LSD than their post-synaptic neurones (Aghajanian and Haigler, 1975; Haigler and Aghajanian, 1974). In a recent study using behavioural measures, it was demonstrated that post-synaptic serotonin receptors display a dramatic tolerance to high doses of LSD following its repeated administration (Trulson, Ross and Jacobs, 1976). Thus, the present study, together with previous studies, suggests that the pre-synaptic receptor (on raphe neurones) differs from the post-synaptic serotonin receptor not only in the differential sensitivity to LSD, but in that tolerance is manifested at the post- but not the pre-synaptic receptor.

Since tolerance to LSD does not occur at the raphe unit level, an alternative explanation must be advanced. If the behavioural effects of LSD are due to the removal of tonic serotonergic inhibition of post-synaptic target neurones, then it is reasonable to assume that tolerance is mediated by a failure of these post-synaptic neurones to increase their activity following repeated LSD administration. Even though the present results indicate that no changes occur in the responsiveness of raphe neurones to LSD in the tolerance condition, the activity of the post-synaptic neurones during tolerance has not been investigated. Thus, the depression of raphe unit activity following LSD may result in no change in the activity of post-synaptic neurones during the tolerance condition. Alternatively, since several recent studies have shown that LSD also acts on central dopamine neurones (Burt, Creese and Snyder, 1976; Pieri, Pieri, Saner, DaPrada and Haefely, 1975; Von Hungen, Roberts, and Hill, 1975; Christoph, Kuhn, and Jacobs, unpublished), tolerance may be mediated by a change in this system.

In conclusion, the profound tolerance which occurs following repeated administration of LSD is apparently not mediated by a change in the responsiveness of serotonin-containing raphe neurones to LSD.

Whether the phenomenon of tolerance to LSD is due to a change at some other level in the serotonergic system is a matter for future investigation.

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Note added in proof: Preliminary data from this laboratory also indicates a lack of tolerance to the depression of raphe unit activity by LSD in freely moving cats.

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