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THE RAPHE NEURONAL SYSTEM AND SEROTONERGIC EFFECTS OF LSD

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Summary—Earlier work from this laboratory had shown that LSD caused significant increases in rat brain serotonin (5-HT). The increase was later localized to a subsynaptosomal fraction consisting largely of synaptic vesicles. However, the source of the increase and the mechanism by which LSD caused the enhanced 5-HT binding or retention had not been elucidated. The present study was undertaken to evaluate the serotonergic effects of LSD following destruction of raphe nuclei with radiofrequency lesions. When LSD was given to animals with large midbrain raphe lesions, it caused significant increases in forebrain or cortical 5-HT up to 48 hr, or 7 days post-lesion, respectively. It was concluded that an intact cell body is not necessary for the expression of the LSD-mediated increases in 5-HT occurring in the nerve-ending. The possible mechanisms by which LSD could act directly at the nerve-ending are discussed.

Since the demonstration of an increase in whole brain 5-hydroxytryptamine (5-HT) following administration of *d*-lysergic acid diethylamide (LSD-25), research has focused on the effects of this drug on the serotonergic system (Freedman, 1961). It was hypothesized that the LSD-induced increase in 5-HT reflected enhanced binding of 5-HT. This assumption was made in part because the increase in 5-HT was confined solely to a crude particulate fraction obtained with high speed centrifugation of the homogenate of rat brain (Freedman, 1961). Subsequent work revealed that the LSD-induced increment in 5-HT occurred in several subcellular fractions (Rosecrans, Lovell and Freedman, 1967; Halaris, Lovell and Freedman, 1972). With improved methodology, however, increments in 5-HT were later detected primarily in the purified nerve-ending fraction (Halaris and Lovell, 1973). Following osmotic disruption of nerve-endings, a 50% increase was obtained in the 5-HT content of a subsynaptosomal fraction consisting largely of synaptic vesicles (Halaris and Freedman, 1977). While these studies determined fairly accurately the subcellular localization of the 5-HT increment after administration of LSD, the source of the increase has presented a puzzle mainly because it occurs during a period of reduced 5-HT metabolism (Freedman, Gottlieb and Lovell, 1970). It is not clear whether the LSD-induced enhanced retention of 5-HT in vesicular fractions is due to: (1) inhibition of release following the cessation of firing of raphe neurons (Aghajanian, Foote and Sheard, 1968), suggesting a site of action of LSD on the raphe soma; (2) to inhibition of 5-HT release associated with the effect of the drug on the raphe axon terminal; or (3) to complex feedback effects mediated through a site of action on the postsynaptic membrane (Freedman and Halaris, 1978).

The present study was designed to evaluate the LSD-induced increase in 5-HT, following destruction of raphe nuclei with radiofrequency lesions. A time course of the effects of the lesion was established. Time points representative of post-lesion degeneration and possible sprouting from partially damaged neurons were included.

METHODS

Animals

Adult male Sprague–Dawley rats (160–180 g) were obtained from King Animal Labs (Oregon, WI). The animals were housed in wire cages with food and water available *ad libitum* in a room maintained at a constant temperature of 24°C with a light–dark cycle of 12 hr (lights on 07.00–19.00 h). Surgical procedures were performed with the animals held in a Kopf rat stereotaxic instrument with the nose clip set at 2.5 mm below interaural zero (Kopf Instruments, Tujunga CA). The animals were anesthetized with ether during surgery, and allowed to recover for several days (as noted in the text) before drug treatment and subsequent sacrifice by decapitation. Brains were removed immediately and dissected on dry ice within 2 min post-mortem. Tissues for analysis were weighed and stored in a cryostatic freezer at −80°C.

Radiofrequency lesions

Radiofrequency lesions were performed with a Radionics lesion generator (model RFG-4) by heating

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the electrode tip to 55°C for 60 sec. The A-P placement was at the juncture of the lambdoidal and saggital sutures. The electrode was lowered, at the midline, in the perpendicular plane to a point 1.0 mm dorsal to the interaural zero for lesioning of the n. centralis superior, and to a point 2.0 mm dorsal for lesioning of the n. dorsalis raphe. Both nuclei were lesioned in all radiofrequency lesion experiments. Sham-operated controls were prepared by opening the skull but sparing the dura.

Histology

Hindbrains from all raphe-lesioned animals were preserved in 10% formalin. Lesion placement was evaluated by random sampling for histological examination. In addition, lesion placements in all brains were evaluated by direct examination of thin coronal sections obtained with a Leitz freezing microtome (model 1310). Specimens reserved for histology were embedded in paraffin, sectioned in the frontal plane at 10 μm thickness, and stained (every fifth section) with cresyl violet.

Drug administration

The drug LSD-25 was dissolved in 0.9% saline and administered at 520 μg/kg (i.p.) Control animals received equivalent volumes of saline. Drug-treated and control animals were sacrificed either 30 or 45 min post-injection as noted in the text.

Assays for 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)

In preliminary studies, 5-HT and 5-HIAA were measured by a fluorometric procedure described previously (Halaris, Jones and Moore 1976). In later experiments, 5-HT and dopamine (DA) were measured electrochemically by a modification of the high pressure liquid chromatography (HPLC) method of Sasa and Blank (1977) as described by Vosmer, DeMet and Halaris (1980). Forebrain samples were obtained by coronal section between the superior colliculi and the mamillary bodies. Cortex was obtained by peeling the cortical structures, including the hippocampus, from the midbrain structures; the corpora striata were included in the sample by transecting the genu of the internal capsule along the length of the thalamus. This dissection was chosen so that areas deriving their serotonergic content purely from the dorsal and median raphe nuclei (neocortex, hippocampus, amygdala, striatum) were included in the sample (Halaris *et al.*, 1976), whereas midbrain structures, which may derive part of their serotonin content from projections of other nuclei (Lorens and Guldberg, 1974), were excluded.

Reagents

Sandoz Pharmaceutical (East Hanover, NJ) supplied LSD. Dopamine 5-HT and 5-HIAA were obtained from Sigma Chemical Co. (St. Louis, MO). Dihydroxybenzylamine (internal standard for HPLC method) was supplied by Aldrich Chemical Co. (Milwaukee, WI). All other reagents used were of analytical grade as supplied by local distributors.

Calculations

Statistical analyses were performed on an HP-55 programmable calculator (Hewlett Packard, Cupertino, CA) using standard programs recommended by the manufacturer.

RESULTS

Localization of lesion placements

The uniform effect of radiofrequency lesions was to destroy the principal components of the midbrain raphe nuclear complex, the nucleus centralis superior and the nucleus raphe dorsalis (Fig. 1). The lesion occasionally extended into other adjacent areas and included portions of the central gray, tegmentum, and median longitudinal fasciculus. In some animals a ventral spreading was observed, which was typically unilateral and included portions of the interpeduncular and pontine nuclei.

Effect of raphe lesions on 5-HT and 5-HIAA in the forebrain

Radiofrequency lesions of the two raphe nuclei resulted in a significant depletion of 5-HT levels in the forebrain. Although 5-HT was unaltered one day after the lesion, on subsequent days, the levels decreased steadily until a 74% depletion was reached on day 21 (Fig. 2). Forebrain 5-HIAA also decreased during the first week post-lesion, but the levels remained un-

Fig. 1. Frontal section through the caudal midbrain demonstrating a combined lesion of the nucleus raphe dorsalis and the nucleus centralis superior. The lesion extends from the aqueduct to the interpeduncular nucleus. The principal components of the midbrain raphe complex are completely destroyed. The lesion extends into adjacent structures, such as the nucleus reticularis tegmenti pontis and the interpeduncular nucleus ventrally, and the central gray dorsally. Cresyl violet strain. × 15.

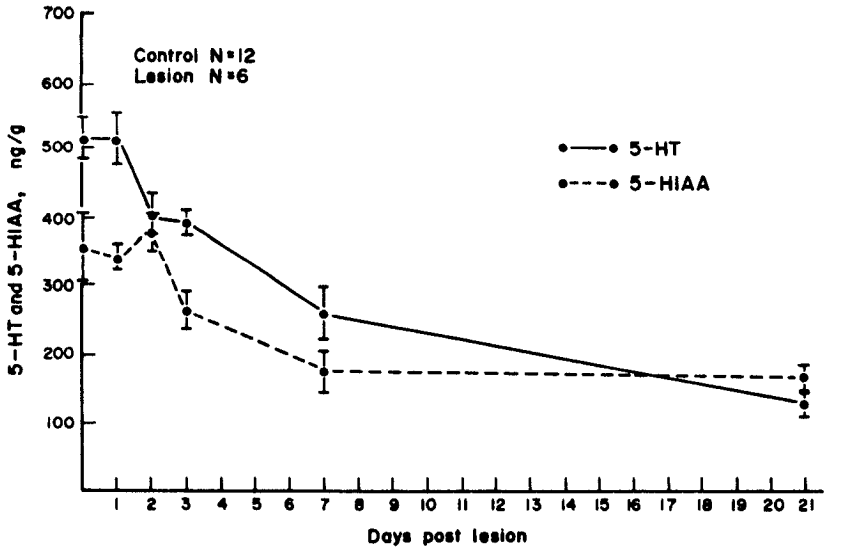

Fig. 2. Time course of the effect of large midbrain raphe lesions on forebrain 5-HT and 5-HIAA. Values are the mean ± SEM for twelve control and six lesioned animals.

altered thereafter. A transient increase in 5-HIAA was seen on day 2, probably accompanying the rapid initial loss of 5-HT.

Effect of LSD on 5HT and 5-HIAA in the forebrain

Administration of LSD (520 μg/kg) to sham-lesioned controls resulted in a 17% increase in 5-HT ($P < 0.005$) in the forebrain and a significant decrease in 5-HIAA ($P < 0.025$). Also, when LSD was given to raphe-lesioned animals it caused a significant increase in 5-HT during the first two days after the lesion (Fig. 3). The increase disappeared on the third day while 5-HT levels were still relatively high (approx. 400 ng/g). A significant decrease in 5-HIAA ($P < 0.025$) was found on the first day post-lesion which was of similar magnitude to that seen in sham-lesioned controls. *d*-Lysergic acid diethylamide (LSD) decreased 5-HIAA also on days 2, 3, and 7 but these

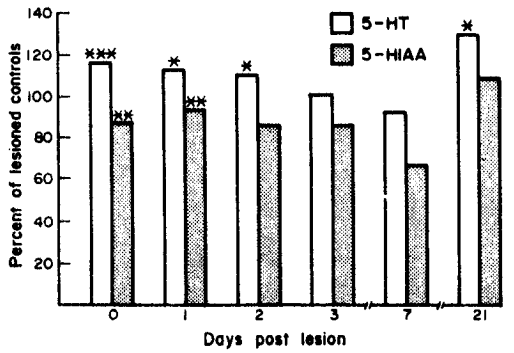

Fig. 3. Time course of the effect of LSD (520 μg/kg, i.p.) on forebrain 5-HT and 5-HIAA. Animals were sacrificed 45 min after drug. Values are expressed as a percentage of lesioned controls. Each bar is the mean of six determinations. Control values were for 5-HT: 511.5 ± 37.2 ($N = 12$); for 5-HIAA: 354.0 ± 40.6 ($N = 12$). *$P < 0.1$; **$P < 0.01$; ***$P < 0.001$.

decreases were not statistically significant due to large variability (Fig. 3); LSD significantly increased 5-HT 21 days after the lesion, but no significant change was found in 5-HIAA. Since levels of 5-HT in the lesioned animals were at their lowest on day 21 (Fig. 2), the reappearance of an increase in 5-HT induced by LSD three weeks after the lesion, might be due to axonal sprouting from partially severed axons. This observation also rules out the possibility that the disappearance of the LSD-induced increment in 5-HT was due to the relatively low 5-HT concentrations on days 3 and 7.

Effect of lesions on 5-HT and DA in the cortex

To maximize the effects of LSD on 5-HT and 5-HIAA in the forebrain in raphe-lesioned animals, the above experiments were repeated using frontal cortex instead of forebrain. Since serotonergic innervation of frontal cortex is derived exclusively from the midbrain raphe nuclei, such measurements should be more specific for the lesioned cells (Halaris *et al.*, 1976). Dopamine levels were also determined in this series of experiments to illustrate the specificity of the results obtained. Figure 4 demonstrates that depletion of 5-HT was progressive over the 3-week period studied. Levels of 5-HT, measured 21 days post-lesion, were 86% depleted indicating that the serotonergic input to the frontal cortex was almost totally destroyed by the lesion. In contrast, DA levels were relatively stable although a slight decrease ($P < 0.05$) was obtained on day 7.

Effect of LSD on 5-HT and DA in the frontal cortex

Five hundred and twenty μg/kg of LSD significantly increased the cortical 5-HT content ($P < 0.005$) in sham-lesioned controls (Fig. 5). Significant increases were also found in lesioned animals 2 and 3

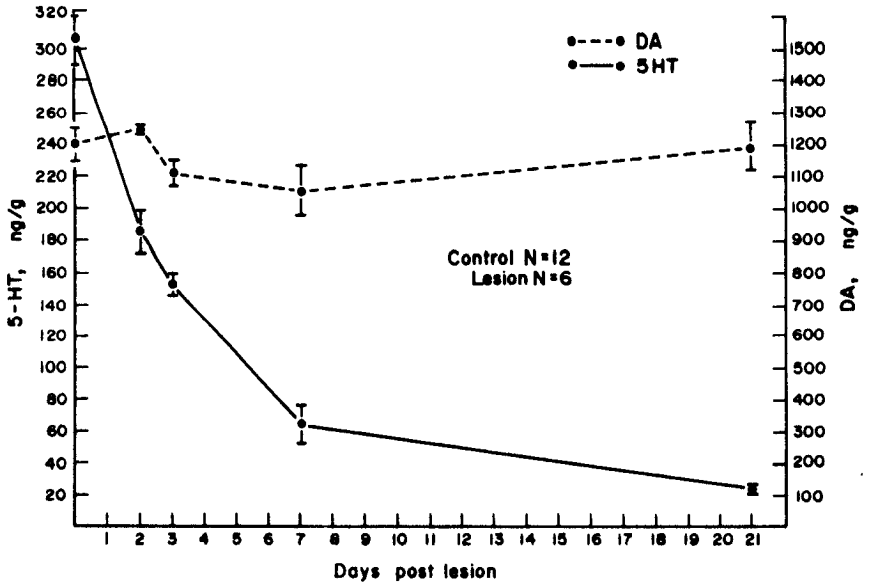

Fig. 4. Time course of the effect of large midbrain raphe lesions on cortical 5-HT and DA. Levels of DA were determined for control purposes. Values are the mean ± SEM for twelve control and six lesioned animals.

days after the lesion ($P < 0.005$). An increase found 1 week after the lesion failed to achieve statistical significance due to the relatively large variability of the samples (see Fig. 4). However, a large LSD-induced increase in 5-HT (relative to control) was observed 3 weeks after the lesion. This increase was similar to the previously observed increase in 5-HT in forebrain and was statistically significant ($P < 0.25$) in spite of the very low levels of 5-HT (Fig. 4). Dopamine levels were not altered significantly by LSD but a trend toward a slight elevation was noted.

DISCUSSION

The present study sought to establish whether the effects of LSD on serotonin levels at the nerve terminal can occur in the absence of intact raphe cell bodies. Destruction of the midbrain raphe complex was achieved by radiofrequency lesions. Administration of LSD to animals with large midbrain raphe lesions resulted in significant increases in 5-HT in the forebrain during the first 48 hr post-lesion. A significant decrease in 5-HIAA was also seen on the first day post-lesion. These results demonstrate that the effects of LSD on both the 5-HT and 5-HIAA levels can occur in the absence of intact midbrain raphe cell bodies. Since the increase in 5-HT in forebrain was moderate, an attempt was made to maximize the effect of LSD by studying a brain region that is richly innervated with raphe axonal projections (Halaris *et al.*, 1976). When frontal cortex was used rather than forebrain, an increase in 5-HT in excess of 30% was obtained up to one week post-lesion. The increase was highly significant ($P < 0.005$) during the first three days after the lesion. Clearly then, LSD can exert serotonergic effects at the nerve terminal, independent of the firing rate or intactness of the raphe soma. It is concluded that the LSD-induced increment in 5-HT in synaptic vesicles is not contingent upon the presence of intact raphe cell bodies.

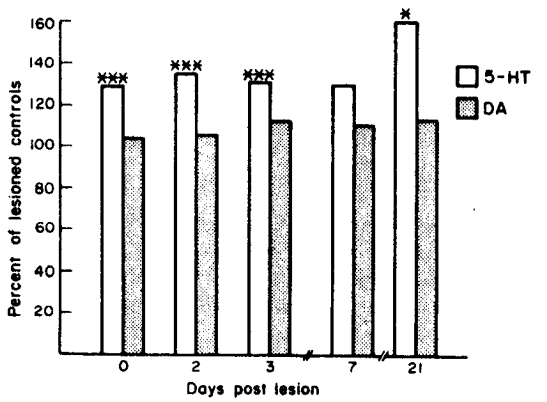

Fig. 5. Effect of LSD (520 μg/kg, i.p.) on cortical 5-HT and DA at various time points after large midbrain raphe lesions. Values are expressed as a percentage of lesioned controls. Each bar is the mean of six determinations. Control values were for 5-HT: 304.8 ± 13.0 ($N = 12$); and for DA: 1,198 ± 52 ($N = 12$). $^{*}P < 0.1$; $^{**}P < 0.01$; $^{***}P < 0.001$.

While these results point to the nerve-ending as the site of action of LSD in inducing increases in 5-HT, the mechanism by which this effect is mediated is not clearly understood. Inhibition of 5-HT release or enhanced retention is a probable mechanism and LSD has been shown to inhibit the release of 5-HT in studies utilizing electrically or K^+-stimulated striatal slices (Chase, Breese and Kopin, 1967; Hamon, Bourgoin, Jagger and Glowinski, 1974) and in studies of the efflux of labelled 5-HT and its metabolite in ventricular fluid (Ghallager and Aghajanian, 1975). The drug also enhanced the retention of intraventricularly

applied ^{3}H-5-HT (Ziegler, Lovell and Freedman, 1973). If LSD acts directly at the nerve-ending to induce retention of 5-HT or to inhibit 5-HT release, binding of LSD should be detectable in areas receiving serotonergic terminal projections.

Recent research on the localization and characterization of LSD binding sites strongly supports the contention that LSD exerts direct effects at the nerve terminal. Tritiated LSD has been found to bind to specific regions in a widespread pattern throughout the brain, with fewer binding sites located in the areas with raphe neurons and a greater amount of binding in areas rich in serotonergic terminal projections (Diab, Freedman and Roth, 1971). Subcellular studies have demonstrated saturable, high-affinity, stereospecific binding which occurred predominantly in crude microsomal fractions (Bennett and Aghajanian, 1974; Bennett and Snyder, 1975; Lovell and Freedman, 1976). There is no evidence that LSD is incorporated into the synaptosomal cytoplasm (Bennett and Snyder, 1975). Rather, LSD binds directly to synaptic membranes. Receptor sites for LSD appear to be present in both pre- and post-synaptic elements (Lovell and Freedman, 1976). However, the localization of tritiated LSD, as demonstrated by autoradiography (Diab *et al.*, 1971), and the fact that raphe lesions failed to alter the binding characteristics of LSD (Bennett and Aghajanian, 1974) make the postsynaptic binding sites appear more significant.

Release of 5-HT could be inhibited by LSD and thus lead to enhanced retention of 5-HT by stimulating a presynaptic 5-HT receptor. Whether this is a specific LSD receptor site remains controversial. Some investigators have postulated that the LSD receptor is a type of 5-HT receptor at which LSD can bind and assume either agonist or antagonist properties (Bennett and Snyder, 1975; Freedman and Halaris, 1978). The evidence is found in the similarity of distribution of both LSD binding sites and sites of high affinity 5-HT uptake, as well as in displacement studies (Bennett and Snyder, 1975; Bennett and Snyder, 1976). The displacement studies show that 5-HT and LSD can displace each other effectively from their respective binding sites. Additionally, LSD is displaced not only by its congeners but also by serotonin antagonists which are presumed to bind to peripheral 5-HT receptors and which mimic the action of 5-HT on raphe cell firing. The identical-receptor theory is also supported by the fact that submaximal doses of 5-HT and LSD act synergistically to inhibit raphe firing activity (Aghajanian, Haigler and Bloom, 1972; Bennett and Snyder, 1975).

Other investigators, however, have postulated the existence of separate receptors for LSD and 5-HT (Fillion, Rousselle, Fillion, Beaudion, Goiny, Deniau and Jacob, 1978). This is based on the complex nature of mutual displacement as well as on the different subcellular distribution of the respective receptors. Whatever the identity of the LSD binding site may be, it is difficult to relate directly these studies to the mechanism of action of multiple drug effects. Peripheral 5-HT antagonists, such as methiothepin, which are effective blockers of the LSD binding sites, do not prevent the raphe cell response to LSD (Freedman and Halaris, 1978). Psychedelic agents, like psilocybin, which inhibit both the firing and the increase whole-brain 5-HT (Freedman, 1961; Aghajanian and Haigler, 1974), bind only weakly to those receptors (Bennett and Snyder, 1975). In fact, the nonpsychoactive LSD congener 2-bromo-LSD (BOL) binds as strongly as LSD but it neither causes nor prevents the action of LSD on raphe firing (Aghajanian *et al.*, 1972). Finally, a postulated feedback mechanism by which activation of postsynaptic receptors should inhibit firing of presynaptic cells is questioned by another study in which surgical transection between pre- and post-synaptic elements failed to abolish the effect of LSD on the presynaptic neurons (Haigler and Aghajanian, 1974).

The results of the present study do not resolve the controversy over the identity of the LSD/5-HT receptor/acceptor site, but they clearly indicate a direct effect of LSD at the level of the presynaptic terminal. The present authors postulate that the LSD-induced enhanced retention of 5-HT is due to the stimulation by LSD of a 5-HT autoreceptor localized at the presynaptic portion of the nerve ending. This receptor may be similar to postsynaptic LSD receptor/acceptor sites or such sites likely to be present on the raphe perikaryon. Stimulation of this autoreceptor probably leads to the inhibition of 5-HT release and this effect is independent of the cell body, as the present results seem to indicate.

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