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Lysergic Acid Diethylamide: Morphological Study of its Effect on Synapses

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Abstract. A morphometric analysis of the effect of LSD on synapses was performed in the habenulae and the interpeduncular nucleus of the frog. The OsO_4-fixed nervous tissue was treated with ethanol phosphotungstic acid (EPTA). Intersection of lattice lines with synapses were counted as a proportion of the total length of lines. LSD-treated frogs had a higher total area of synaptic contact than control frogs. Exocytosis profiles were observed only in LSD-treated frogs. Other qualitative changes in the ultrastructural characteristic of synapses were appreciable after LSD administration.

Key words: Quantitation – LSD – Limbic structures – Synaptic changes.

Lysergic acid diethylamide (LSD) is a potent hallucinogenic substance. The literature regarding the sites and mode of action of LSD in the central nervous system (CNS) is often controversial. In fact, neurochemical, electrophysiological, and behavioral studies indicate that LSD interacts with the serotonergic, noradrenergic, and dopaminergic systems, LSD acts either as an agonist or antagonist, and it has contrasting effects (Martin and Sloane, 1977 for review). However, there is a general agreement that LSD interacts with neurotransmitters, thus implying that this psychotomimetic drug affects the site of neurotransmission, i.e., the synapse.

The present paper is part of an extensive morphological investigation on the effect of LSD on CNS structures. We have already reported some ultrastructural modifications induced by LSD on the brain of the frog (Kemali and Kemali, 1979a, b); the reversible production of lipid-like droplets and modifications of the ciliary apparatus in certain zones of the ventricular surface. We also found an enhancement of the synaptic active site resulting in the appearance of exocytotic profiles.

It has been underlined that the morphologic approach to the dynamic aspect of synaptology should be quantitated to reveal whether or not real differences exist between experimentally treated and control animals (Vrensen and De Groot, 1973). A quantitative ultrastructural study was therefore undertaken to try to correlate the effect of LSD to synaptic changes in the habenulae and the interpeduncular nucleus.

These regions were chosen because they are part of the limbic circuit and are interconnected to the site of origin of the mesolimbic and mesocortical dopaminergic systems (Cuello et al., 1978). It has been suggested that these pathways are involved in some psychiatric disorders (Carlsson, 1976).

Materials and Methods

Three *Rana esculenta* male frogs (one being a control), weighing approximately 20 g were used. The animals were anesthetized with MS 222 (0.5 g in 1000 ml) and a cut made in the midline of the skin of the head. LSD (1 mg/kg in 0.25 ml H_2O) was injected into a large blood vessel running over the lateral surface of the skull of two frogs. Their survival time was 2 h.

The three frogs were treated by the ethanol phosphotungstic acid (EPTA) method for the selective staining of the synaptic membranes (Bloom and Aghajanian, 1968) after being fixed in OsO_4 so that the synaptic vesicles were also shown.

The habenular nuclei and the interpeduncular nucleus were easily dissected out and horizontal cuts made, starting directly under the pia, from the dorsal side of the brain for the habenulae, and from the ventral side for the interpeduncular nucleus. Identical areas were sampled in all animals. Ten random pictures were taken at regular intervals along the cerebral structures investigated.

To quantify the surface area of synaptic contacts in control and LSD-treated frogs, we followed the methodological criteria described by Altschuler (1979) for the morphometric analysis of synaptic changes. The surface area of synaptic contacts per volume, based on the intersection of lattice lines with synapses, was calculated using the appropriate formula reported by Vrensen and De Groot (1973). The

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mean surface density for each cerebral zone was determined in control and LSD-treated frogs.

The exocytosis profiles were observed using the same micrographs greatly enlarged.

Results

The surface synaptic density in LSD-treated frogs was higher than in the control. It was 52% higher in the habenular nuclei and 45% higher in the interpeduncular nucleus.

Figure 1 shows the ultrastructure of synapses in control and in LSD-treated frogs. The density of synaptic vesicles in some terminals is low (compare the three synaptic terminals in Fig. 1 d). This could be due to the type of fixation (combination of OsO_4 and EPTA), as also in the control frog some synaptic terminals have a low density of vesicles (Fig. 1 a). It can be seen that in LSD-treated frogs the vesicles are often aligned in rows which seem to follow a preferential pathway towards the presynaptic membrane, onto which they closely adhere (arrows in Figs. 1 b and d). On the contrary, in the control frog, in the same type of synaptic terminal, the vesicles are divided from the presynaptic membrane by well-defined dense projections (Fig. 1 a).

The arrow in Fig. 1 c, indicates an exocytosis profile. These were observed only in LSD-treated frogs thus confirming previous observations on aldehyde-fixed material (Kemali and Kemali, 1979 a, b). Exocytosis is a morphologic event which occurs as a response to a stimulation. They have been reported at the level of the neuromuscular junction of the frog following various stimulations (Clark et al., 1972; Heuser et al., 1979) and in vitro preparation of frog brain after the administration of potassium chloride (Van Harreveld and Trubatch, 1975).

Discussion

Our data suggest that administration of LSD might result in a pharmacological stimulation involving the synaptic vesicles. In addition, our results have demonstrated quantitative synaptic differences between control and LSD-treated frogs. The increased surface area of synaptic contacts may reflect an increase in the

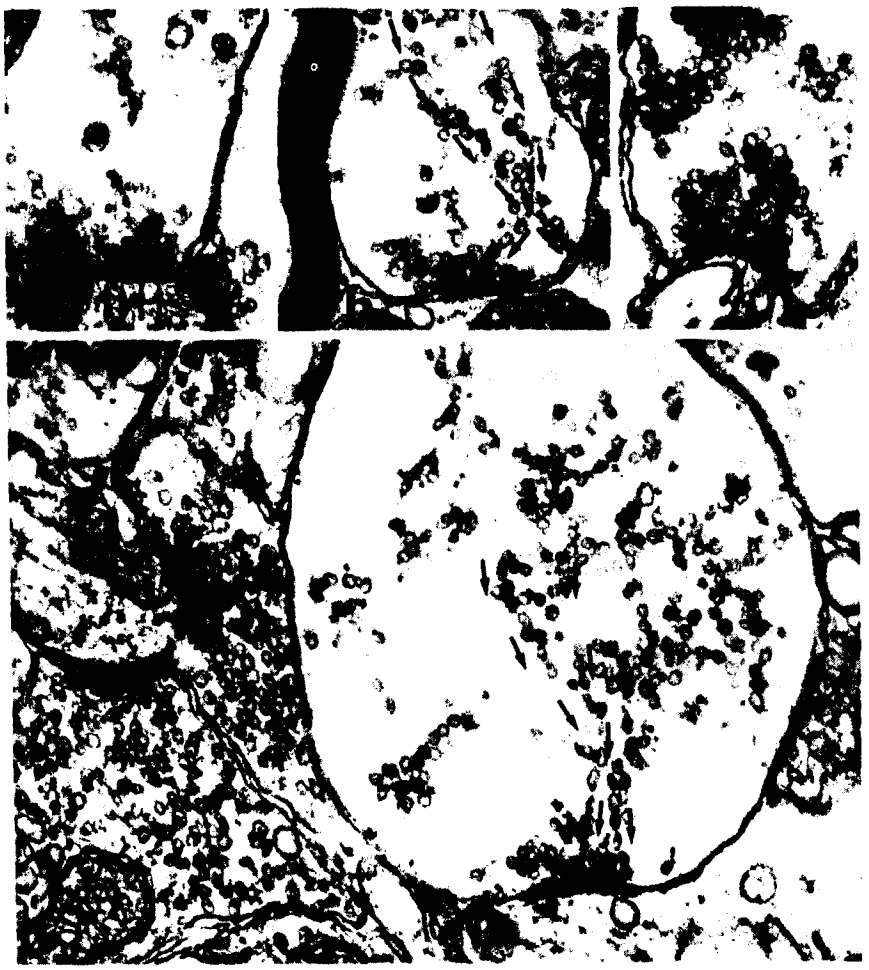

Fig. 1
Synapses in the interpeduncular nucleus of the control frog (**a**) and LSD-treated frogs (**b**, **c**, and **d**): (**a**) The arrows in the post-synaptic site indicate the dense projections appearing in the presynaptic membrane (× 35,500); (**b**) synapse with a low density of vesicles. The arrows are disposed along the pathways followed by the vesicles. On the left, a myelinated axon (× 35,500); (**c**) the arrow shows an exocytosis profile (× 45,000); (**d**) two types of synaptic terminals with different vesicle densities. The arrows are disposed along pathways followed by vesicle rows (× 41,000)

number of synapses or an increase in the dimensions of existing synaptic contact.

In conclusion, it seems that the administration of LSD induced synaptic changes in two structures of the limbic circuit.

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