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OBSERVATIONS ON CENTRAL NERVOUS SYSTEM STRUCTURES FOLLOWING THE ADMINISTRATION OF A PSYCHOTOMIMETIC SUBSTANCE (1)

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The incidental discovery that Lysergic-Saüre-Diäthylamid (LSD) is a potent hallucinogenic substance has opened new prospectives in the study of some aspects of mental illnesses.

The fact that a chemical compound, synthetized in the laboratory, has psychotomimetic effects and has a chemical configuration similar to that of a neurotransmitter — serotonin — present in the central nervous system, has stimulated several lines of research, from behaviour to electrophysiology and from neurochemistry to psychopharmacology, regarding the effect of this drug on man and on experimental animals.

It has been demonstrated that LSD has many sites of action in the central nervous system (on the serotoninergic, nor-adrenergic, dopaminergic systems), but its mechanism of action is still poorly understood (for review see MARTIN and SLOANE, 1977).

The present report describes the effect of the intravenous administration of LSD on the cerebral structures of the frog *Rana esculenta*. Frogs were anaesthetized with MS-222 and injected with a single dose of LSD (1 mg/kg in 0.25 ml of distilled water) into a large blood vessel running over the lateral surface of the skull. After periods of survival of 2 and half hours and 7 days, the frogs were fixed by perfusion with a mixture of aldehydes and the dissected brain tissues were post-fixed in osmium tetroxide and observed at the light and electron microscope.

RESULTS

A survey, at the light microscope, of the entire brain cut in semi-thin sections, 2 microns thick, revealed 2 and half hours after the injection the

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presence of morphological abnormalities in the ventricular surface system of LSD-treated frogs compared with the untreated ones. These abnormalities consisted of huge dark spherical droplets particularly abundant in the cerebral aqueduct (fig. 1).

When observed at the electron microscope, the huge droplets appeared to lack a limiting membrane and had the ultrastructural characteristics of lipid droplets (fig. 2, a-d). These intracytoplasmatic inclusions were also observed in the endothelium of blood vessels (fig. 2, a) and in neurons of the periventricular zones (fig. 2. b). In addition, the electron microscope revealed that the ciliary apparatus of the ventricular system was also involved showing an increase in the number and length of the ependyma cilia (fig. 2, c). These are reversible events since they disappear after 7 days of survival following drug administration.

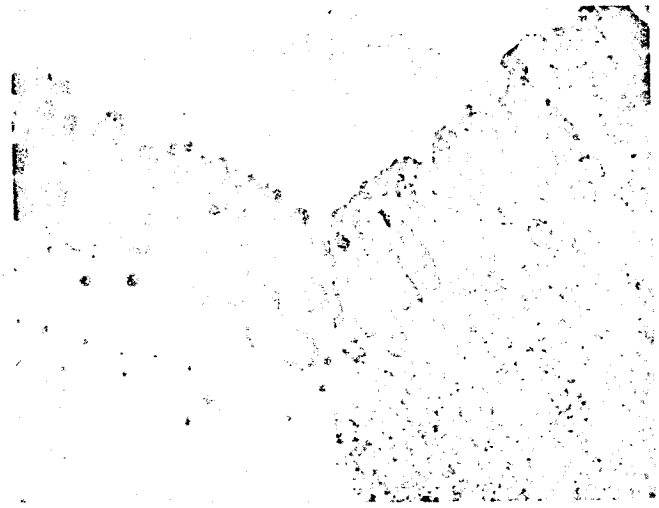

FIG. 1. — Semi-thin sections of an LSD treated (on the left) and an untreated frog (on the right) at the level of the cerebral aqueduct. Note on the LSD treated specimen large, spherical dark granules within the ependyma. 500 X.

With reference to the possible effect of LSD on synapses, we observed an enhancement of the « active zone » resulting in the appearence of exocytotic profiles, that is fusion between the membranes of the synaptic vesicles and the pre-synaptic membrane which is the transmission site of the synapse. The exocytosis is usually considered the morphologic response to a stimulation of any sort, as demonstrated at the level of the neuromuscular junction of the frog stimulated with black widow spider venom (CLARK *et al.*, 1972).

Figure 3 shows one of these « stimulated » synapses occurring in the habenular nuclei which are structures belonging to the limbic system.

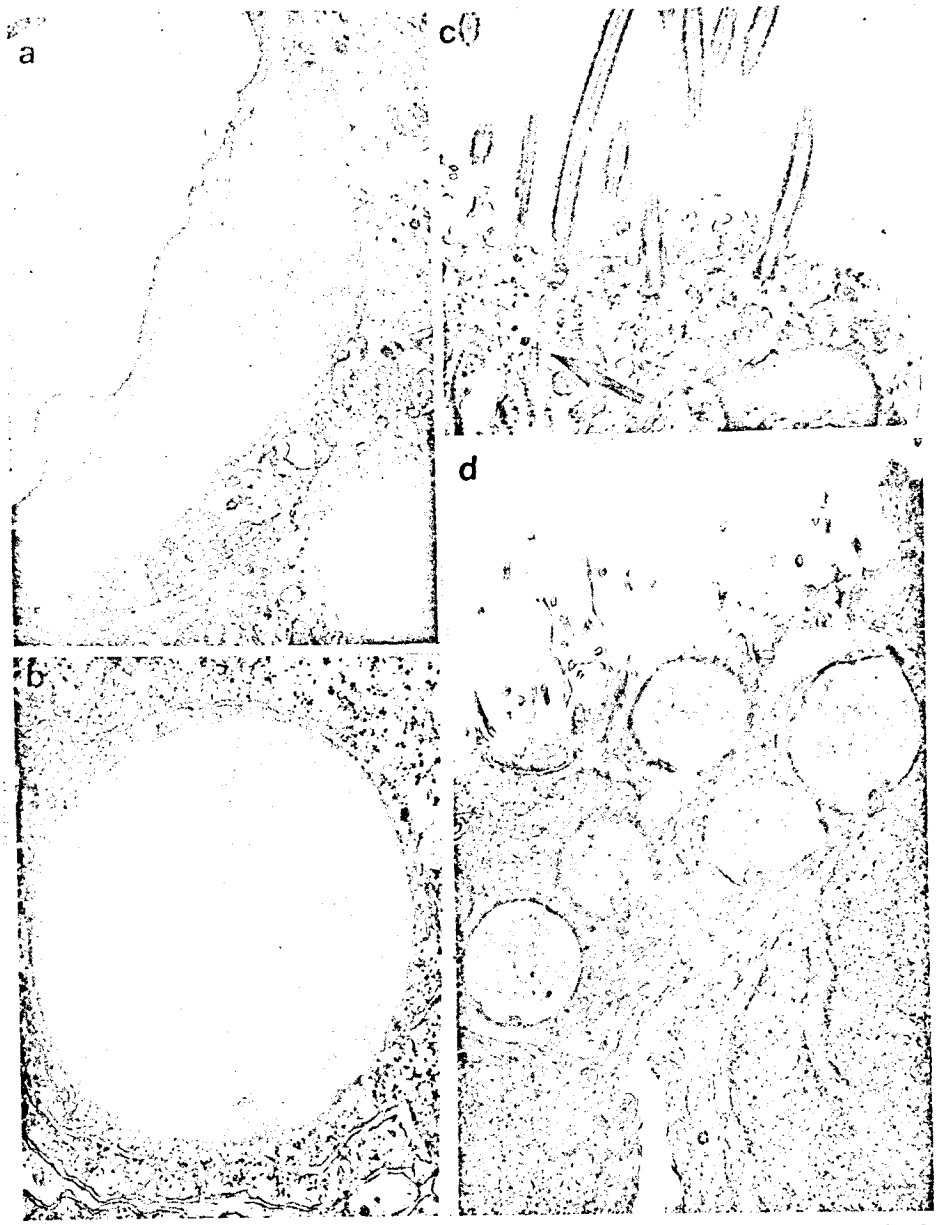

FIG. 2. — A) Blood vessel of the habenular nuclei of an LSD treated frog showing lipid-like droplets within the endothelium. A huge droplet is appearing in the right low corner of the picture. 7,800 X. B) Large lipid-like droplet included within the cytoplasm of a neuron in the telencephalon. The droplet is devoid of a limiting membrane. 27,500 X. C) Telencephalic ependymal cell showing numerous long cilia, extremely long ciliary rootlets (arrow) and a droplet on the right. 16,500 X. D) Several lipid-like droplets in the cytoplasm of ependyma cells of the cerebral aqueduct. 14,850 X.

DISCUSSION

Recent findings have demonstrated that in mammals the habenular nuclei receive inputs of both striatal and limbic origin (HERKENHAM and NAUTA, 1977) and therefore appear to be one of the few brain regions in which these two mayor parallel systems are know to converge. Furthermore, the habenulae are interconnected both with the raphae nuclei (AGHAJANIAN and WANG, 1977) and with the site of origin of the dopaminergic mesocortical and mesolimbic systems, known as zone A 10. This connection is made through substance "P" containing habenular efferents (CUELLO *et al.*, 1978) and probably dopaminergic-containing habenular afferents (BJÖRKLUND, 1978). The habenular nuclei of the frog have the same connections that are found in higher forms as a study based on the axonal transport of horseradish peroxidase has demonstrated (KEMALI *et al.*, in preparation).

The administration of LSD probably results in a pharmacological stimulation involving one type of synapse — at least in the habenulae — those with small, clear, spherical vesicles. This is of note because we have demonstrated at the electron microscope a surprising variety of synaptic vesicles in the habenular nuclei of normal frogs (KEMALI, 1976; KEMALI and GUGLIELMOTTI, 1977; KEMALI, 1977).

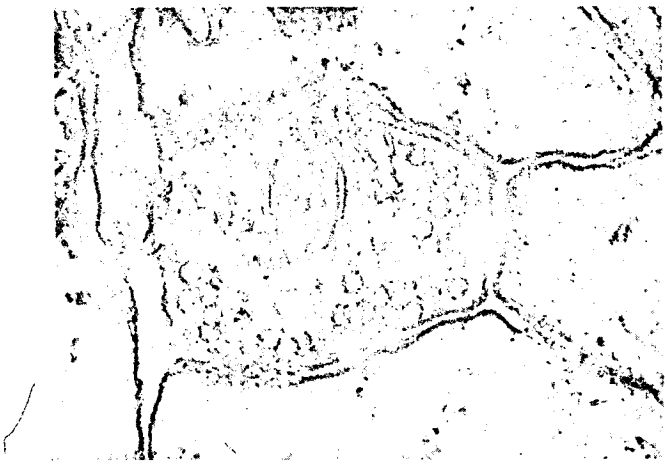

FIG. 3. — Synapse of the habenular nuclei following intravenous administration of LSD. Note the exocytotic profile. 80,000 X.

The interpretation of the morphological alterations reported after the administration of LSD go beyond the scope of this paper and would be only on a speculative basis. We wish only to underline that lipid droplets have been reported after intense sympathetic activity (NUNEZ *et al.*, 1975) and an anomalous formation of cilia has been stimulated by the administration of MAO

inhibitors in the habenular nuclei (MILHAUD and PAPPAS, 1968), structures rich in serotonin.

In conclusion, we have the morphologic evidence that the intravenous administration of LSD may cause reversible alterations of selected zones of the ventricular surfaces and the involvement of the synaptic apparatus in a structure of that portion of the limbic system which in man represents the phylogenetically oldest portion of this circuit. Further studies are necessary in order to see if monoamines and/or indoleamines might be involved in the production of the events reported here.

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SUMMARY

Following the administration of 1 mg/Kg of LSD by intravenous route, we observed in the brain of the frog Rana esculenta after 2 hours and half of survival, the presence of huge droplets on the ependymal surface and periventricular zones. They are particularly abundant at the level of the cerebral acqueduct and for their ultrastructural features are comparable to lipid droplets. This event is reversible. Modifications have also been noted in the ependymal ciliary apparatus. Exocytotic figures have been observed at the level of synaptic terminals of the habenular nuclei bearing small, clear, spherical vesicles.

RIASSUNTO

In seguito alla somministrazione endovena di una singola dose di LSD (1 mg/Kg) sono state osservate, nel cervello della *Rana esculenta*, dopo due ore e mezzo di sopravvivenza, delle grosse gocce sulle superfici ependimali e nelle zone periventricolari. Tali gocce, che al microscopio elettronico sono risultate prive di membrana limitante, sono probabilmnete di tipo lipidico. Esse sono particolarmente abbondanti a livello dell'acquedotto cerebrale. Anche l'apparato ciliare ependimale è risultato particolarmente stimolato. Dopo sette giorni di sopravvivenza tali eventi sono quasi completamente regrediti. Sono state inoltre osservate delle figure di exocitosi a carico di sinapsi dei nuclei abenulari aventi vescicole piccole, sferiche e chiare.

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