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Modification of the Pigment Screening of the Frog Retina Following Administration of Neuroactive Drugs

M. KEMALI, N. MILICI* AND D. KEMALI*

*Instituto Cibernetica CNR, 80072 Arco Felice (Naples), Italy and *1st Psychiatric Clinic, University of Naples, 80138 Naples, Italy*

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Pigment screening (PS) occurs in the retina of lower vertebrates and consists of the bidirectional migration (vitreally or sclerally) of melanin granules into processes of the pigment epithelium that extend between photoreceptors, in response to changes in the illumination conditions. We have studied the effect of some neuroactive drugs on the PS of frogs maintained under cyclic lighting conditions or dark-adapted.

The drugs, administered intravenously were: lysergic acid diethylamide (LSD), mescaline, d-amphetamine, the LSD analogue lisuride and the LSD derivative 2-bromolysergic acid (BOL). All the drugs used – with the exception of mescaline – modify the bidirectional migration of the pigment induced by the two illumination conditions in a different way. This suggests that in general these substances interact in some way with those processes which normally produce the well-defined PS pattern.

It has been possible to discriminate two opposite effects on the retinal PS induced by two chemically related substances (LSD and lisuride) only one of which (LSD) has potent hallucinogenic properties.

Key words: frog retina; pigment screening; neuroactive drugs; hallucinogens.

1. Introduction

Pigment screening (PS) is a phenomenon occurring in the retina of lower vertebrates following illumination (Nguyen-Legros, 1978) and consists of the dispersion of melanin granules into processes of the pigment epithelium which extend between photoreceptors (vitreal migration). In darkness the phenomenon is reversed, the same pigment aggregating within the cell body of the pigment epithelium (scleral migration).

Although the mechanism regulating the pigment screening is not fully understood, it is presumed that it is activated by photoreceptors since these are the main elements influenced by light. From the anatomical point of view, photoreceptors and pigment epithelial cells in lower vertebrates do not show any structural sign of connection so it has been suggested that some kind of humoral factor might be involved such as melatonin for the aggregation and amino acids like taurine for the dispersion of pigment (Flight, 1979).

Melatonin – a serotonin derived compound – is the principal hormonal agent of the pineal (Wurtman, Axelrod and Kelly, 1968). However, it has been recently localized immunohistochemically in the outer nuclear layer of the retina by Vivien-Roels, Pevet, Dubois, Arendt and Brown (1981) who suggested that it is synthesised by this structure.

Moreover, besides the occurrence in the retina of putative neurotransmitters such as dopamine, acetylcholine, serotonin and certain neuroactive amino acids (Graham, 1974), a number of neuropeptides have been demonstrated there by means of immunohistochemical techniques (Eskay, Long and Iuvone, 1980; Stell, Marshak, Yamada, Brecha and Karten, 1980).

Please address reprint requests to: M. Kemali, M.D., Instituto Cibernetica, 80072 Arco Felice (Naples Italy.

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In the present study, which is part of a broader study into the interaction between drugs and nervous system, we used the PS of the frog retina to see if and how its pattern is modified by the administration of neuroactive drugs. These were: lysergic acid diethylamide (LSD), mescaline, d-amphetamine, the LSD analogue lisuride and the LSD derivative 2-bromo-lysergic acid (BOL).

In addition, we aimed at seeing if the PS pattern was affected differently by structurally related drugs, one of which (LSD) has a potent visual hallucinogenic effect while of the others, Lisuride has not and BOL's might have psychogenic properties. (Hoffmeister and Stille, 1982).

2. Material and Methods

A total of 24 frogs, four being controls, of the species *Rana esculenta* were used. One group was kept under a normal day/night light cycle. One group was dark-adapted. Each experiment was carried out on two specimens, for each illumination condition. The drugs were administered to unanaesthetized frogs by intravenous injection as detailed elsewhere (Kemali and Kemali, 1979) in amounts of 1 mg/kg (LSD, BOL, lisuride, d-amphetamine) and 15 mg/kg (mescaline) in 0·25 ml of distilled water. Control animals, kept under the same lighting conditions as the experimental frogs, received 0·25 ml of saline by the same route. All the steps concerning dark-adapted frogs were carried out in dim red light. The histological procedures were carried out at the same time in the morning hours for all the specimens.

After a survival time of 2 hrs following drug administration, the frogs were decapitated and the eyes enucleated and opened by means of ophthalmic scissors at the level of the ora serrata. The lens was then extracted and the whole retina with the attached pigment epithelium left within the sclera, was fixed in 10% formalin for several days.

The retinae were then dehydrated, embedded in tissue matt, cut in serial sections, 15 μm thick, and stained according to the Nissl method. Sections were made in a rostro-caudal direction perpendicular to the long axis of the eye. Sections from equivalent zones of the retina were compared.

3. Results

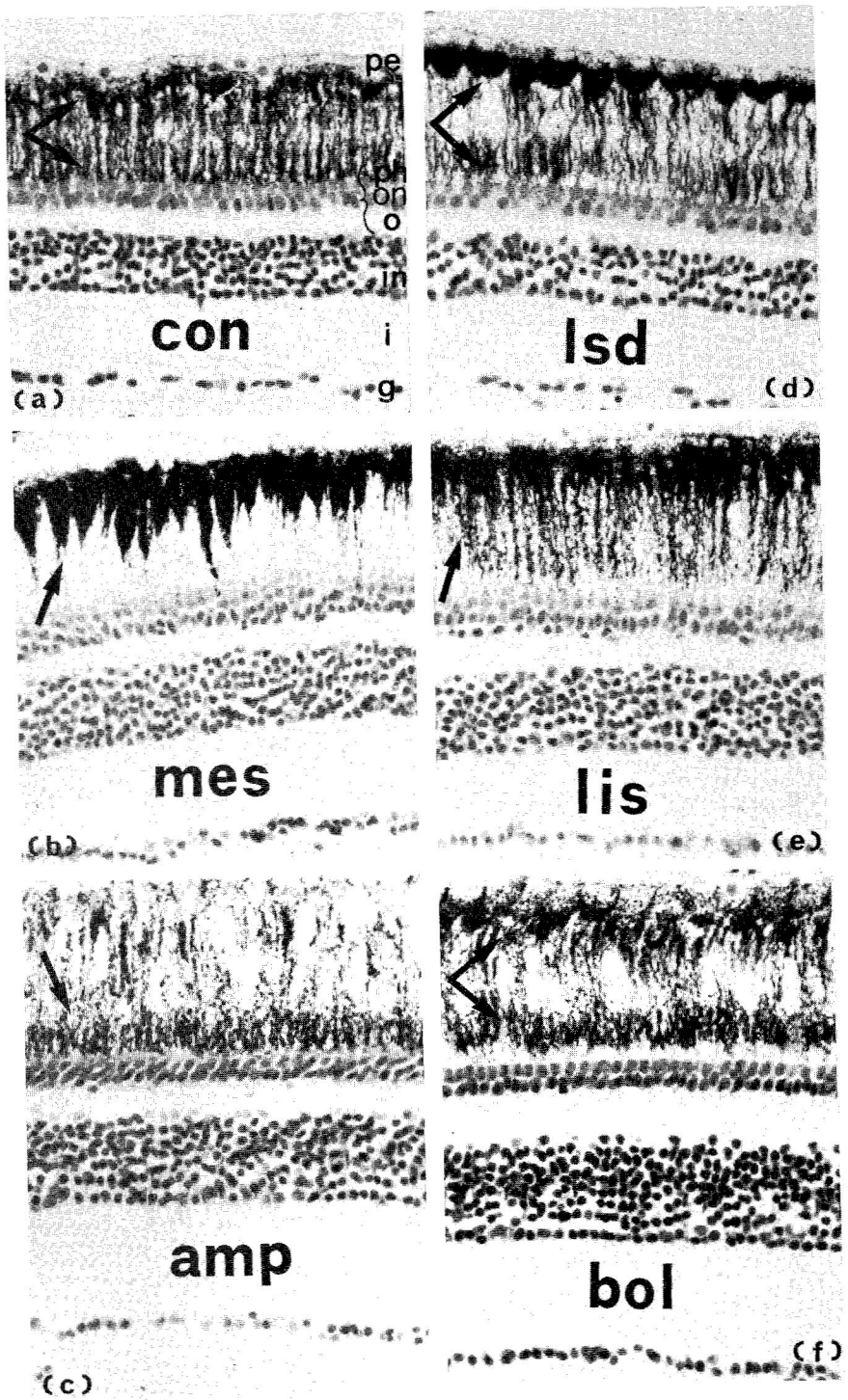
In control frogs kept in cyclic lighting conditions, the major part of the pigment migrates vitreally within the apical processes of the pigment epithelial cells which surround the photoreceptor outer segment [arrows in Fig. 1(a)].

In contrast, the pigment migrates sclerally in the dark-adapted control frog and becomes aggregated within the cell body of the pigment epithelium [arrow in Fig. 2(a)].

Cyclic lighting conditions

The administration of LSD [Fig. 1(d)] and of BOL [Fig. 1(f)] induces a partial migration of pigment vitreally which resembles that of the control eyes. However, with LSD a quantity of pigment is strikingly aggregated sclerally and gives the epithelial cells the shape of a tea-cup. Aggregation of pigment in both directions is greater in BOL-treated animals than in the controls but does not show the aggregation in the pigment epithelial cells on the scleral side as does occur after the administration of LSD.

FIG. 1. Sections of the retina from frogs kept in cyclic lighting conditions. The arrows indicate the direction of migration and aggregation of the pigment. The layers of the retina are shown in the first panel (a) for normal frog retina (g, ganglion cells; i, inner plexiform; in, inner nuclear; o, outer plexiform; on, outer nuclear; ph, photoreceptors; pe, pigment epithelium). The sections illustrate the modification of the pigment screening after administration of mescaline (b), amphetamine (c), LSD (d), lisuride (e) and BOL (f). Con, control; mes, mescaline; amp, amphetamine; lis, lisuride; lsd, LSD; bol, BOL. ×250.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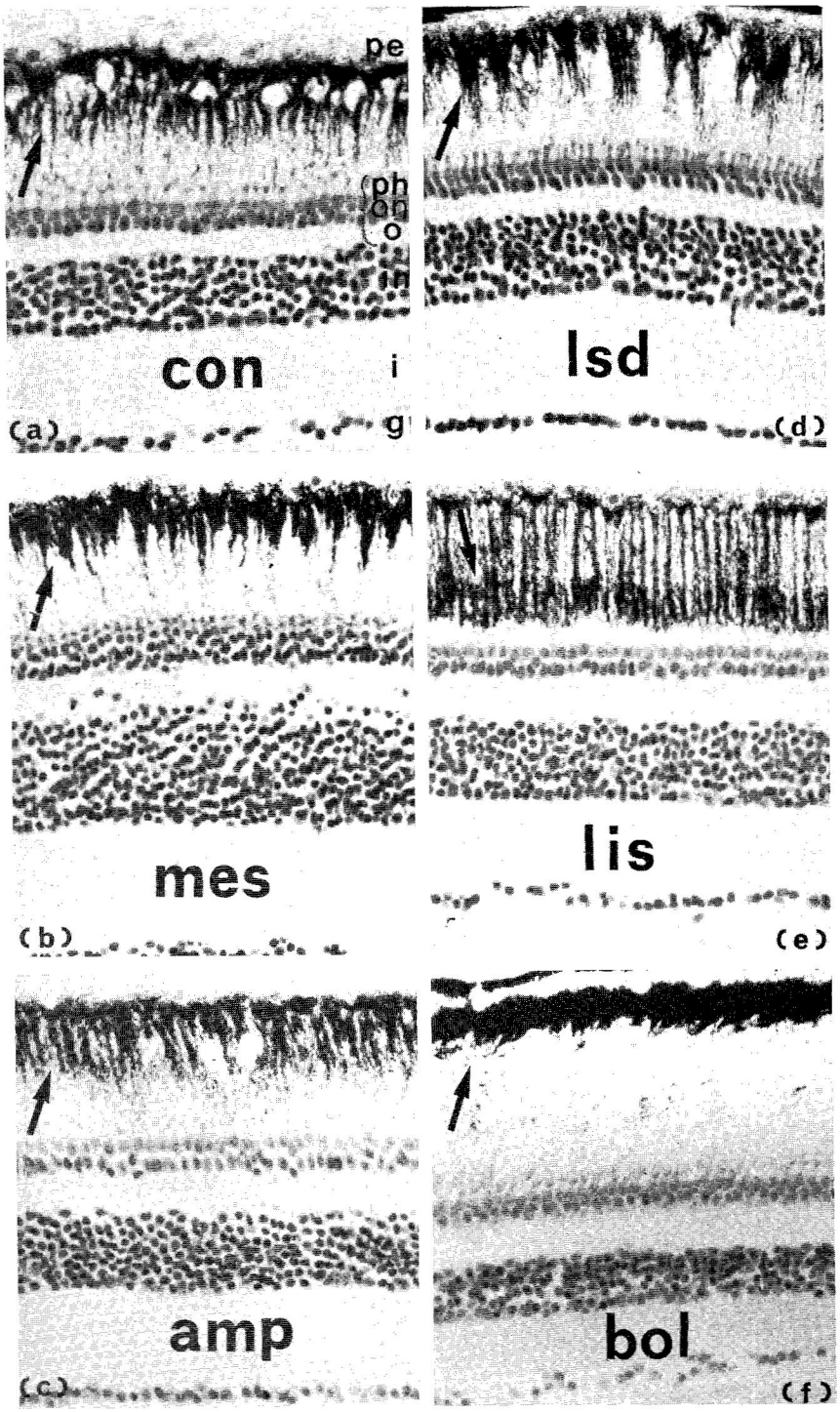

FIG. 2. Sections of the retina from dark-adapted frogs. ×250. Arrows and lettering as in Fig. 1.

The administration of mescaline [Fig. 1(b)] and of lisuride [Fig. 1(e)] induces a migration and aggregation of pigment granules sclerally which gives the epithelial cells the shape of a narrow V after administration of mescaline [arrow in Fig. 1(b)] and a shapeless massive dark accumulation after administration of lisuride [arrow in Fig. 1(e)].

The administration of amphetamine induces a greater migration of pigment granules vitreally [arrow in Fig. 1(c)] than in controls, while the scleral epithelial surface is nearly devoid of such pigment.

Dark-adaptation

The administration of LSD, mescaline, amphetamine and BOL, although with slight individual variations, induces a migration and accumulation of pigment in the scleral direction [arrows in Fig. 2(d), (b), (c) and (f) respectively] resembling, in a general way, the PS pattern of the controls. In contrast, the administration of lisuride induces a pigment migration in the opposite direction into the apical fringes of the epithelial cells between the photoreceptors [arrow in Fig. 2(e)].

On summarizing the results, it is apparent that an obvious bidirectional migration of pigment (sclerally or vitreally depending in the light conditions) occurs in the retina of normal frogs. After the administration of lisuride the direction of pigment migration is reversed in comparison to control [see Figs 1 and 2(a) and (e)]. After LSD, BOL and amphetamine this change was less obvious. The pigment aggregation towards the scleral surface induced by the administration of mescaline is the only one to remain unchanged in the two illuminated states [see (b) in Figs 1 and 2].

4. Discussion

The results reported here show that the drugs used in this study modify in various ways and with their own particular variations the pattern of the PS of the frog retina. It is presumed that they affect, by an unknown mechanism, those factors which normally produce aggregation or dispersion of melanin granules within the pigment epithelium.

The chemical structure of the drugs may be of importance in their interaction with melanin (Atlasik, Stepien and Wilczok, 1980). The drugs used in this investigation can be grouped as those structurally related to catecholamines (mescaline and amphetamine) and those structurally related to serotonin (LSD, lisuride, BOL) (see Hoffmeister and Stille, 1982). However, LSD, mescaline and amphetamine are psychotomimetic agents, mescaline and LSD being psychedelic drugs which interfere mainly with visual perception. (Lisuride is not psychotomimetic and the psychotogenic action of BOL is controversial.)

That it is possible to discriminate opposite morphological effects occurring in the PS of dark-adapted frog retina induced by LSD, a psychotomimetic drug with potent hallucinogenic properties, and lisuride, a LSD analogue but which lacks such properties, is of particular interest.

Since this effect is found only in dark-adapted frogs, we presume that illumination conditions must have an influence in modifying the action induced on PS pattern by these compounds which are chemically related (LSD and lisuride) but have different properties. Such influence might be indirect since it is known that illumination conditions affect neurotransmitters turnover. Light stimulates dopamine synthesis in the retina (Iuvone, Galli, Garrison-Gund and Neff, 1978), influences the release of

melatonin (Wurtman, Axelrod and Kelly, 1968) and affects differentially the levels of GABA and taurine (Nishimura, Ida and Kuriyama, 1981); are all substances which might be involved in the PS pattern of the normal frog retina. Mescaline, however, is the only drug whose effects are not influenced by illumination conditions of the retina since it induces the same PS pattern in light and dark-adapted frogs. It is evident that the two psychedelic compounds, LSD and mescaline, act by a different mechanism or on different components.

Our study does not clarify by what mechanism the drugs used modify the PS of the frog retina. However, we would like to suggest that drugs known to produce visual hallucinations may involve primarily or secondarily the retina.

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