Melatonin and LSD Induce Similar Retinal Changes in the Frog

M. Kemali, N. Milici, and D. Kemali

Introduction

This work is based on histological examination with the light microscope of Nissl-stained frogs' retinae following melatonin and lysergic acid diethylamide (LSD) administration. In a previous paper (Kemali et al. 1983) we have demonstrated with the light microscope that administration of LSD induced in the frog retina a modification of the pigment screening (PS) pattern through some unknown mechanism.

PS is a phenomenon encountered in the retina of low vertebrates and involves the migration of melanin granules from the retinal pigment epithelium in response to illumination changes. In the light, melanin granules disperse along the pigment epithelium processes, which protrude among the outer segments of the photoreceptors. In the dark, on the other hand, melanin granules aggregate within the cell body of the pigment epithelium (for review see Nguyen-Legros 1978).

In another earlier paper (Kemali et al. 1984) we have demonstrated that the administration of dopaminergic agents to the frog did not induce any substantial modifications of this PS pattern, suggesting that dopamine is probably not directly involved in this retinomotor phenomenon.

The aim of the present investigation was to see how the PS of the frog retina was affected by the administration of the indoleamine melatonin. This interest was prompted by the observations that melatonin is found not only in the pineal body, but also in the retina (Bubenik et al. 1978; Gern and Ralph 1979; Hamm and Menaker 1980; Pang et al. 1982; Leino 1984), that melatonin receptors have been demonstrated both on cells of the neural retina and on cells of the pigment epithelium (Gern et al. 1981), and that in the frog *Rana esculenta,* it has been demonstrated that the neural part and the pigment epithelium of the retina are involved in methoxyindole synthesis with a diurnal rhythmicity (Flight et al. 1983). However, we have examined the action not only of exogenous melatonin, but also of LSD, on the retina of light- and dark-adapted frogs and have compared the results produced on the PS by the two compounds.

Methods

Fourteen frogs (four being controls) of the species *Rana esculenta* were utilized. A group of seven was kept under continuous illumination; another group of seven was dark-adapted. A single dose of melatonin (0.7 mg/kg) was injected intraperitoneally into two light-adapted and two dark-adapted frogs, and a single dose LSD (1 mg/kg) was injected intravenously into one light-adapted and one dark-adapted frog. Two frogs maintained under the same illumination conditions were used as controls and were injected with a single dose of saline.

From the Istituto di Cibernetica del CNR, Naples (M.K.), and the First Psychiatric Clinic, University of Naples (N.M., D.K.), Naples, Italy.
Address reprint requests to Dr. M. Kemali, Istituto di Cibernetica, CNR, 80072 Arco Felice, Naples, Italy.
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Two light-adapted frogs and two dark-adapted frogs received the same amount of melatonin (0.7 mg/kg) intraperitoneally every day at midday for 15 days running. Two other frogs, one light- and one dark-adapted, were treated equally, but with saline, and served as controls.

The frogs were killed by decapitation 2 hr after the administration of the single doses of melatonin and LSD and 2 hr after the last injection of the fortnightly melatonin treatment.

The retinae were dissected out rapidly. The eyes were opened by means of ophthalmic scissors at the level of the ora serrata. The lenses were then extracted, and the whole retina, with the attached pigment epithelium left within the sclera, was fixed in 10% formalin for several days. The dark-adapted frogs were processed in dim red light.

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

Results

The action of different illumination conditions on the PS pattern of the retina of the control frogs that received a single injection of saline is shown in parts a and b of Figure 1, which are respectively, the retinae, of one light-adapted frog and one dark-adapted frog. From the illustrations it is apparent that light induces the migration of the major part of the melanin pigment within the apical processes of the pigment epithelial cells, which protrude among photoreceptors (Figure 1a). On the other hand, the pigment aggregates toward the layer of the pigment epithelium cell perikaryon when in the dark (Figure 1b). The results with the light- and dark-adapted control frogs that received the 15-day treatment with saline were similar to those illustrated in parts a and b, respectively, of Figure 1.

As already reported (Kemali et al. 1983), and as repeated here experimentally, in the light, the administration of LSD induces a remarkable aggregation of pigment within the cell body of the epithelial cell, which assumes, in all cases, a characteristic shape resembling a teacup (Figure 1c). An interesting parallel is found by administering a single dose of melatonin, which induces a PS pattern in light-adapted frogs (Figure 1e) similar to that induced by LSD. The administration of melatonin for 15 days under light conditions produces a decrease of pigment and a slight accumulation thereof at the scleral portion of the epithelial pigment cell (Figure 1g).

In the dark, LSD (Figure 1d) and melatonin (Figure 1f), in single doses, yield a similar pattern of massive accumulation of pigment at the portion of the process close to the pigment cell perikaryon, which is not very different from the pattern in the retina of control dark-adapted frogs (Figure 1b). Melatonin administered for 15 days to dark-adapted frogs induces a rather homogeneous distribution of pigment along the processes of the pigmented epithelial cells, with a slight accumulation of melanin in the central part of the pigment cell elongations (Figure 1h). The administration of melatonin, both as a single injection and as a 15-day course, was repeated twice in both light- and dark-adapted frogs, with respective results as illustrated in Figure 1(e–h).

Discussion

The results presented in this article demonstrate that the administration of two indole-related substances, melatonin and LSD, induced modifications of the PS of the frog retina. In particular, when a single dose of melatonin or LSD is injected, the PS modified pattern, both in light- and dark-adapted frogs, is the same for the two substances.

Melatonin is the main indolic hormone released by the pineal organ and is currently considered to be antigonadotrophic in function. LSD is another indole compound with potent psychotomimetic properties that acts on the nervous system with a mechanism of action not yet fully understood. Although morphological and functional similarities between the retina and the

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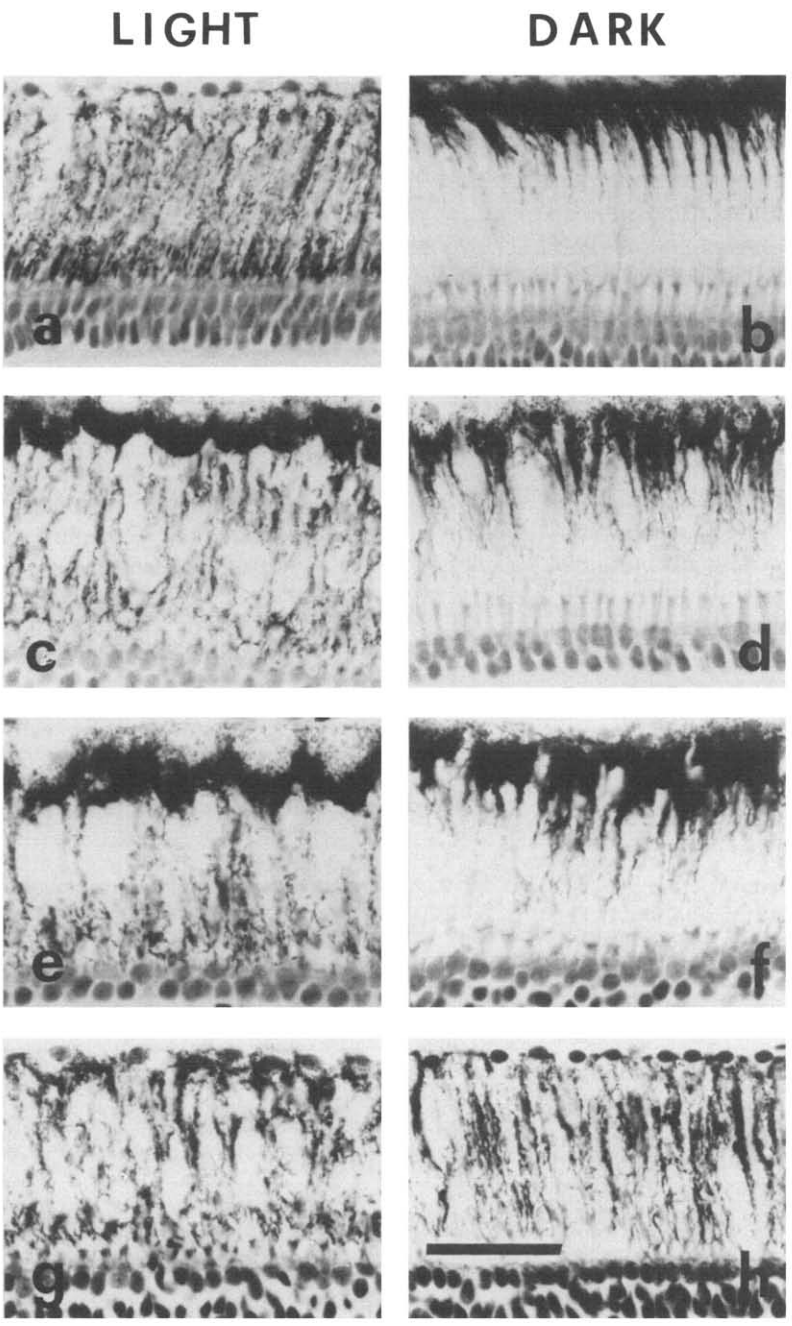

Figure 1. Sections from the retinae of eight frogs stained by the Nissl method. Calibration bar = 50 μ. On the left are sections from the retina of light-adapted frogs. On the right are sections from the retina of dark-adapted frogs. Only the outer nuclear, the photoreceptor, and the pigment epithelial layers appear, as only the pigment screening phenomenon is of interest in this study. (a, b) Sections of the retinae of two control frogs injected with saline; (c, d) sections of the retinae of two frogs injected with a single dose of LSD; (e, f) sections of the retinae of two frogs injected with a single dose of melatonin; (g, h) sections of the retinae of two frogs injected with melatonin for 15 days running.

pineal organ of lower vertebrates have been demonstrated (Flight 1979), the finding of melatonin in the retina of various animals (Bubenik et al. 1978; Gern and Ralph 1979; Hamm and Menaker 1980; Pang et al. 1982) and in man (Leino 1984) is an intriguing occurrence that raises many questions regarding its possible significance. It has been proposed (Flight 1979; Gern 1981) that melatonin produced by retinal photoreceptors might regulate PS in the retina of lower vertebrates, whereas it is known that in the pineal and in the retina, the melatonin content is high in the dark and low in the light (Pang et al. 1982). In agreement with this, we observed that under illumination, when melatonin production is inhibited, the exogenous administration of a single dose of melatonin induces an aggregation of melanin granules in the pigment epithelium cells (Figure 1e). In the dark, when the content of melatonin is high, there is a probable synergism of effects between endogenous and exogenous melatonin, resulting in the pattern shown in Figure 1f. The repeated injection of melatonin for 15 days gave results different from those obtained after the administration of a single dose of melatonin. We may suppose that the contrasting results that ensue when a single dose or when several successive doses are administered may be due to a differential sensitization of the melatonin receptors shown to be present both on the neuronal and on the pigment epithelium cells of the retina (Gern et al. 1981). In addition, it has been demonstrated (Chen 1981) that pineal melatonin can exert opposite effects on the reproductive axis depending on whether the injections are large or small.

However, the function of retinal melatonin is not only to regulate the PS, but also to inhibit the release of dopamine from the retina (Dubocovich 1983), and it is thus responsible for plasma melatonin levels, at least in trout (Gern et al. 1978). In a previous study, we ruled out the direct involvement of dopamine in the phenomenon of PS (Kemali et al. 1984). As the precursor of melatonin is serotonin (Axelrod 1978), one cannot disregard the possibility that this indole or a complex of indoles, recently found to be synthesized in the frog's retina (Flight et al. 1983), might interact in some way with those processes that normally produce the well-defined PS pattern. Whether this is a direct action or occurs through the involvement of one of the numerous transmitters or peptides that have been found recently in the retina is impossible to say.

The mechanism of action of melatonin and LSD on the induction of the PS patterns on the frog retina remains unknown. However, it is of interest that a single dose of melatonin and a single dose of LSD induced the same modification of the PS in the frog retina. This may indicate that a possible functional relationship between different indole-related compounds is reflected in this retinomotor phenomenon.

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