Comp. Biochem. Physiol. [C] 51: 59-62 (June 1) 1975

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Comp. Biochem. Physiol., 1975, Vol. 51C, pp. 59 to 62. Pergamon Press. Printed in Great Britain

COLOR CHANGES INDUCED BY CERTAIN INDOLE ALKALOIDS IN THE FIDDLER CRAB, *UCA**

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(*Received* 5 *November* 1974)

Abstract—1. Lysergic acid diethylamide (LSD) evokes distal retinal pigment light adaptation and erythrophoric pigment concentration.

2. The erythrophoric responses to LSD are not dependent on the amount of light striking the rhabdoms in the eyes.

3. Isolysergic acid amide and psilocin evoke erythrophoric pigment concentration in intact fiddler crabs but not in eyestalkless crabs nor *in vitro* in isolated legs. It was already known that LSD evokes such pigment concentration in intact crabs only.

4. Of the three alkaloids used, LSD was the most potent while psilocin was the least potent in evoking the observed effects on the erythrophores.

5. These indole alkaloids do not stimulate the erythrophores directly. Their actions appear to be mediated by the neuroendocrine system.

INTRODUCTION

LYSERGIC acid diethylamide (LSD) evokes color changes in the crustaceans *Palaemon squilla* (Östlund & Fänge, 1956), *Palaemon paucidens* (Aoto, 1963), *Uca pugilator* (Fingerman & Rao, 1970) and *Cambarellus shufeldti* (Rao & Fingerman, 1975). In view of the observation that LSD evoked leucophoric pigment concentration in intact *P. paucidens* but not in eyestalkless animals, Aoto (1963) suggested that LSD causes the retinal pigments to further uncover the rhabdoms, resulting in an increase in the amount of light being perceived. Somehow this increased amount of light impinging on the rhabdoms would then produce the pigment concentration. Whether this hypothesis is correct still remains to be determined. Previous experiments of Fingerman & Rao (1970) revealed that LSD causes erythrophoric pigment concentration in intact fiddler crabs but does not evoke pigment concentration in the erythrophores of eyestalkless fiddler crabs.

In view of the report of Aoto (1963) cited above, experiments were designed to determine whether the color changes induced by LSD in intact crabs are always tied to light-adapting movements of the distal retinal pigment in *Uca*. Furthermore, the relative efficacy of certain indole alkaloids, psilocin, D-isolysergic acid amide and LSD in evoking erythrophoric pigment concentration in *Uca* was determined.

* This investigation was supported by Grant GB-27497 from the National Science Foundation to M. F. and by a faculty research fellowship awarded to K. R. R. by the Research Council, University of West Florida.

MATERIALS AND METHODS

Drugs

D-Lysergic acid diethylamide (LSD, Sandoz), D-isolysergic acid amide (Sandoz) and psilocin (Sandoz) obtained through the courtesy of the National Institute of Mental Health, U.S.A., were used in this study. Serial dilutions were prepared in crustacean physiological saline (Pantin, 1934) immediately before use.

Crabs

Specimens of the fiddler crab designated as the dark patch variant of *Uca pugilator* (Rao & Fingerman, 1968) were used in this study. In view of its morphological and physiological (Rao & Fingerman, 1968), biochemical (Fielder *et al.*, 1971a) and behavioral specificities, the dark patch variant is being described as a new species of *Uca* (Barnwell, personal communication). Therefore, the crabs are referred to herein merely as *Uca*. Specimens used in this study were either collected from Sabine Island, Florida, or supplied by the Gulf Specimen Company, Panacea, Florida.

Assay for distal retinal pigment migration

The position of the distal retinal pigment within the eyes of *Uca* was determined using the method of Fingerman (1970). The crab was immersed, ventral surface down, in a dish of sea water kept on the stage of a stereoscopic dissecting microscope. The following two measurements were made with the aid of an ocular micrometer and transmitted light: (1) the width of the widest portion of the translucent distal portion of the eye along a line at a right angle to the pigmented portion of the eye and (2) the width of the eye along the line of first measurement from the distal edge of the cornea to the opposite edge of the eyestalk. The ratio:

$$\frac{\text{width of the translucent area}}{\text{total width}}$$

is expressed as the Distal Pigment Index (DPI). Using the method described in detail by Fielder *et al.* (1971b), the effects of injection of 0·5 and 5 μg LSD/0·05 ml saline per crab on the distal retinal pigment were determined.

Assay for erythrophoric pigment concentration

In vivo assays were performed on three types of crabs: (1) intact crabs, (2) crabs blinded by covering both eyes with a non-toxic black acrylic paint and (3) eyestalkless crabs. All the test solutions were injected in a dose of 0·05 ml/crab. The controls received the same volume of saline alone.

In vitro assays were carried out with isolated legs as described earlier (Rao & Fingerman, 1970). The erythrophores were staged following the scheme of Hogben & Slome (1931) whereby stage 1 represents maximal pigment concentration, stage 5 maximal dispersion and stages 2, 3 and 4 the intermediate conditions. All experiments dealing with the chromatophores were carried out during the daytime under a light intensity of 370–430 lx and at a temperature of 22–25°C.

RESULTS

Action of LSD on the distal retinal pigment

The effects of injection of two dosages of LSD on the position of the distal retinal pigment of *Uca* are shown in Fig. 1 where each data point is the average

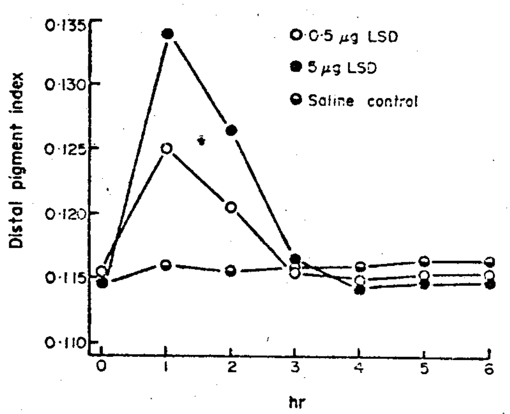

Fig. 1. Responses of the distal retinal pigment in the fiddler crab, *Uca*, to LSD.

value for ten crabs. It is clear from the figure that LSD evoked light adaptation of the distal retinal pigment. The amplitude of the light-adapting response evoked by 5 μg of LSD was greater than that evoked by 0·5 μg of LSD. LSD did not cause migration of the retinal pigment to a dark-adapted position.

Action of LSD on erythrophores of normal and blinded fiddler crabs

In order to determine whether the alteration in the retinal pigment caused by LSD injection has any influence on the accompanying color change, LSD was injected into normal and blinded crabs. The results are shown in Fig. 2 where each data point is

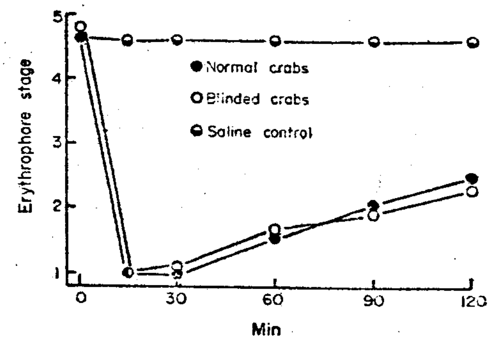

Fig. 2. Responses of the erythrophores in intact and blinded (eyes covered with black paint) fiddler crabs to LSD.

the average value for twenty crabs. There is no difference between the amounts of erythrophoric pigment concentration evoked by LSD in normal and blinded crabs.

Comparison of the effects of psilocin, isolysergic acid amide and LSD

In order to determine the relative efficacy of the three alkaloids the following dosages were injected into normal crabs that had been on a black background for 2 hr, prior to the experiment: 1, 10, 25 and 50 μg psilocin/crab; 0·1, 1, 5 and 10 μg isolysergic acid amide/crab; and 0·1, 1, 5 and 10 μg LSD/crab. Each of the dosages was tested on ten to fifteen crabs. The results of injection of representative dosages, shown in Fig. 3, reveal that all three drugs evoke erythrophoric pigment concentration in normal crabs. At each of the dosages tested, LSD was the most potent while psilocin was the least potent in producing pigment concentration in the erythrophores. For example, the red pigment concentration evoked by 0·1 μg LSD is of comparable magnitude to that evoked by 50 μg of psilocin.

Assay of psilocin and isolysergic acid amide on eyestalkless crabs and isolated legs

In order to compare the mode of actions of psilocin and isolysergic acid amide with that of LSD, solutions of 10 μg psilocin/0·05 ml and 10 μg isolysergic acid amide/0·05 ml were each tested on ten eyestalkless crabs and ten legs isolated from intact

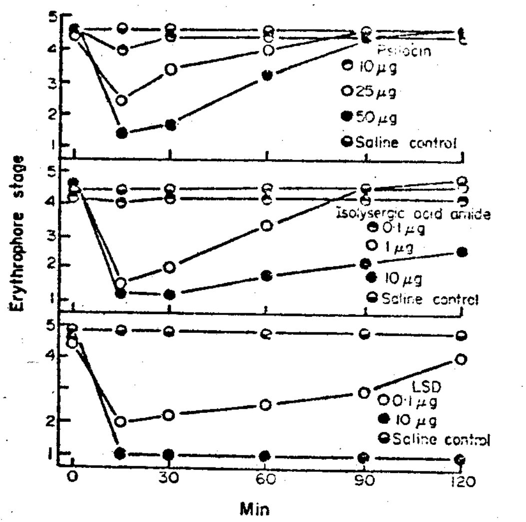

Fig. 3. Responses of the erythrophores in intact fiddler crabs to psilocin, isolysergic acid amide and LSD.

crabs on a black background. In all of these assays, psilocin and isolysergic acid amide failed to evoke erythrophoric pigment concentration.

DISCUSSION

Hormonally controlled movements of retinal pigments within the compound eyes of crustaceans contribute to the mechanical adaptation of the eyes to changes in light intensity (Kleinholz, 1936; Fingerman, 1970; Fielder *et al.*, 1971b). The light adaptation of the distal retinal pigment produced by LSD (Fig. 1) is comparable to that evoked by injection of an eyestalk extract (Fielder *et al.*, 1971b). The experimental protocol did not permit us to determine whether the observed light adaptation that occurred in the LSD-injected crabs was due to a direct effect of the drug on the retinal pigment or was mediated by the distal retinal pigment light-adapting hormone.

There appears to be no relationship between the effects of LSD on the retinal pigment and the erythrophores. The finding that LSD induces essentially the same amounts of erythrophoric pigment concentration in intact and blinded crabs (Fig. 2) indicates that the observed color changes are not due to alterations in the amount of light striking the rhabdomeres.

It is known that the general hallucinogenic effects produced by LSD are also produced by psilocin, psilocybin and mescaline. These drugs differ from one another primarily in their time course of action (Levine, 1969). For example, the hallucinogenic effects produced by LSD last longer (and are of greater magnitude) than those obtained with psilocin. In this regard it is of interest to note that the crustacean erythrophoric pigment-concentrating potencies of these two drugs exhibit a similar relationship. The amplitude and duration of the red pigment concentration evoked by LSD are much greater than with psilocin (Fig. 3).

Just as was observed with LSD in previous experiments (Fingerman & Rao, 1970), we observed in the present experiments that isolysergic acid amide and psilocin evoked eyrthrophoric pigment concentration in intact crabs but not in eyestalkless crabs nor *in vitro* in isolated legs. Therefore, it can be concluded that these alkaloids do not directly affect the erythrophores. Isolysergic acid amide and psilocin presumably exert their effects primarily on the eyestalk neuroendocrine system to cause the release of red pigment-concentrating hormone as shown to be true for LSD (Fingerman & Rao, 1970).

Note added in proof.

The species of fiddler crab used in this investigation has been given the name *Uca panacea*. (Novak A. & Salmon M. (1974) *Uca panacea*, a new species of fiddler crab from the Gulf Coast of the United States. *Proc. Biol. Soc. Wash.* 87, 313–326).

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Key Word Index—Color change; distal retinal pigment; erythrophores; fiddler crab; indole alkaloids; isolysergic acid amide; lysergic acid diethylamide; psilocin; *Uca*.