Comp. gen. Pharmac., 1970, 1, 341–348

ACTION OF BIOGENIC AMINES ON CRUSTACEAN CHROMATOPHORES

III. ANTAGONISM BY LYSERGIC ACID DIETHYLAMIDE OF THE EFFECT OF SEROTONIN ON COLOUR CHANGES IN THE FIDDLER CRAB, *UCA PUGILATOR**

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(*Received* 28 *April*, 1970)

ABSTRACT

1. Lysergic acid diethylamide (LSD) evokes strong concentration of the erythrophoric pigment in intact fiddler crabs, *Uca pugilator*, but does not evoke pigment concentration in the erythrophores of eyestalkless crabs or legs isolated from intact crabs.

2. LSD has no effect on maximally concentrated erythrophoric pigment.

3. LSD inhibits the red pigment dispersing action of serotonin (5-hydroxytryptamine), but does not antagonize the action of red pigment dispersing hormone from the eyestalk.

4. LSD does not stimulate the erythrophores directly; its action is mediated by the neuroendocrine system in the crab.

LYSERGIC acid diethylamide (LSD) causes blanching of some vertebrates and darkening of others. For example, in the frog, *Rana temporaria*, LSD produces melanin dispersion (Kahr and Fischer, 1957) whereas in two other amphibians, *Xenopus laevis* (Burgers, Leemreis, Dominiczak, and van Oordt, 1958; Burgers and Imai, 1962; Burgers, Zandee, van Bakel, Hillemans, and van Oordt, 1962) and *Bufo arenarum* (Iturriza and Koch, 1964), it causes melanin concentration. In amphibians LSD appears to exert its effect indirectly through the pituitary rather than directly on the melanophores. Hypophysectomized specimens of *Rana temporaria* do not respond to LSD; the drug presumably exerts its effect in this frog by stimulating release of the melanin-dispersing hormone, intermedin, from the pituitary gland, whereas it has been shown in *Xenopus laevis* and *Bufo arenarum* that LSD prevents the release of intermedin from the pituitary gland, ultimately resulting in blanching of the skin. In contrast to these and other investigations of the effect of LSD in vertebrates (*see* review of Burgers, 1966) very little is known of its actions in crustaceans. Östlund and Fänge (1956) reported that LSD in doses of 10^{-4} and 10^{-3} g. per ml. evoked a slight pigment concentration in the large erythrophores of eyestalkless specimens of the prawn, *Palaemon squilla*. Aoto (1963), however, found that LSD evokes both pigment concentration and pigment dispersion in the erythrophores as well as in the leucophores of *Palaemon paucidens*, depending upon the state of the pigment at the time of injection. The mode of action of LSD in these prawns is uncertain, and so far LSD has not been tested on any brachyuran.

The present report is the first detailed account of the mode of action of LSD on colour changes in a brachyuran. In a previous publication (Rao and Fingerman, 1970) we have shown that 5-HT (5-hydroxytryptamine, serotonin) evokes pigment dispersion in the erythrophores of the fiddler crab, *Uca pugilator*, and that this action rather than being a direct one on the erythrophores as is that of the red pigment dispersing hormone (RPDH) is instead mediated by the

* This investigation was supported by Grant GB-7595X from the National Science Foundation.

neuroendocrine system of this crab. Therefore, it seemed worth while to determine the interaction between LSD, 5-HT, and RPDH in order to evaluate further the mode of action of these substances on crustacean colour changes.

MATERIALS AND METHODS

Drugs

D-Lysergic acid diethylamide (LSD-25; Sandoz), obtained through the courtesy of the National Institute of Mental Health, and serotonin creatinine sulphate (5-hydroxytryptamine creatinine sulphate, 5-HT; Sigma) were used in this study. Serial dilutions of the compounds were prepared in crustacean physiological saline (Pantin, 1934) immediately before use.

Hormones

A fraction with red pigment dispersing activity, RPDH, was isolated from the eyestalk as described earlier (Rao and Fingerman, 1970). An acetone-soluble fraction of freshly dissected eyestalks prepared according to the method of Rao, Fingerman, and Bartell (1967) was used as a source of red pigment concentrating hormone (RPCH).

Assays

Specimens of the fiddler crab, *Uca pugilator*, supplied by the Gulf Specimen Company, Panacea, Florida, were used in this investigation. Intact crabs adapted to a white or black background for at least 2 hours were used to assay for red pigment dispersing and concentrating activities respectively. In the crabs adapted to a white background the pigment in the erythrophores was concentrated, while in those adapted to a black background it was dispersed. Eyestalkless specimens of the light patch variant of *Uca pugilator* with their erythrophoric pigment fully dispersed (Rao and Fingerman, 1968) were also used in assays for red pigment concentrating activity.

All the test solutions (except in the assays for the chromatophorotropic activity of blood for which details are given in the text below) were injected in a dose of 0·05 ml. per crab. The controls received saline alone. The erythrophores were staged according to the method of Hogben and Slome (1931) whereby stage 1 represents maximal pigment concentration, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions.

In vitro assays were performed as described earlier (Rao and Fingerman, 1970). All experiments were carried out during the daytime under a light intensity of 377 metre-candles and at a temperature of 22–24° C.

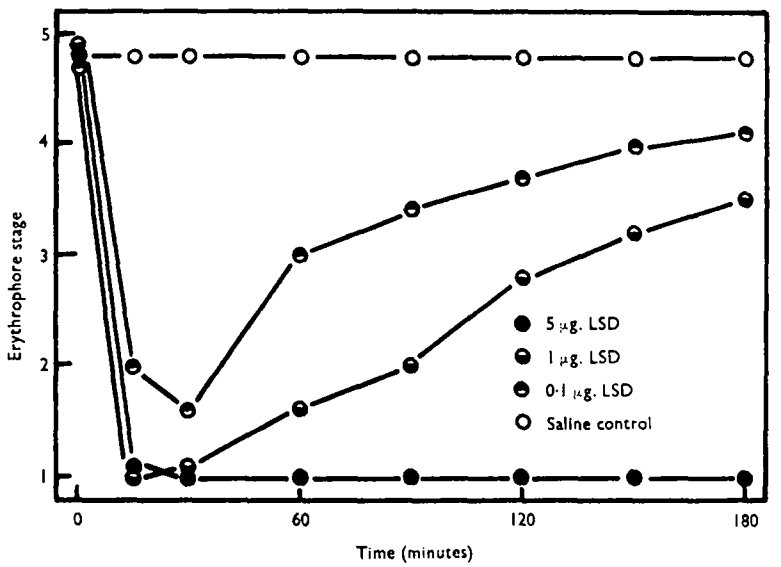

FIG. 1.—Effects of injection of LSD on the erythrophores of intact *Uca pugilator* adapted to a black background.

RESULTS

Action of LSD on Intact Crabs

To determine whether LSD has any effect on the erythrophores of intact specimens of *Uca pugilator* various dosages (0·1–5 μg. per crab) were injected into groups of crabs adapted to black and to white backgrounds.

The results of injection of LSD into crabs adapted to a black background are shown in *Fig.* 1, in which each curve is based on data from 15 individuals. It is clear that LSD evoked pronounced red pigment concentration. However, the maximally concentrated erythrophoric pigment of intact crabs adapted to a white background showed no response to LSD.

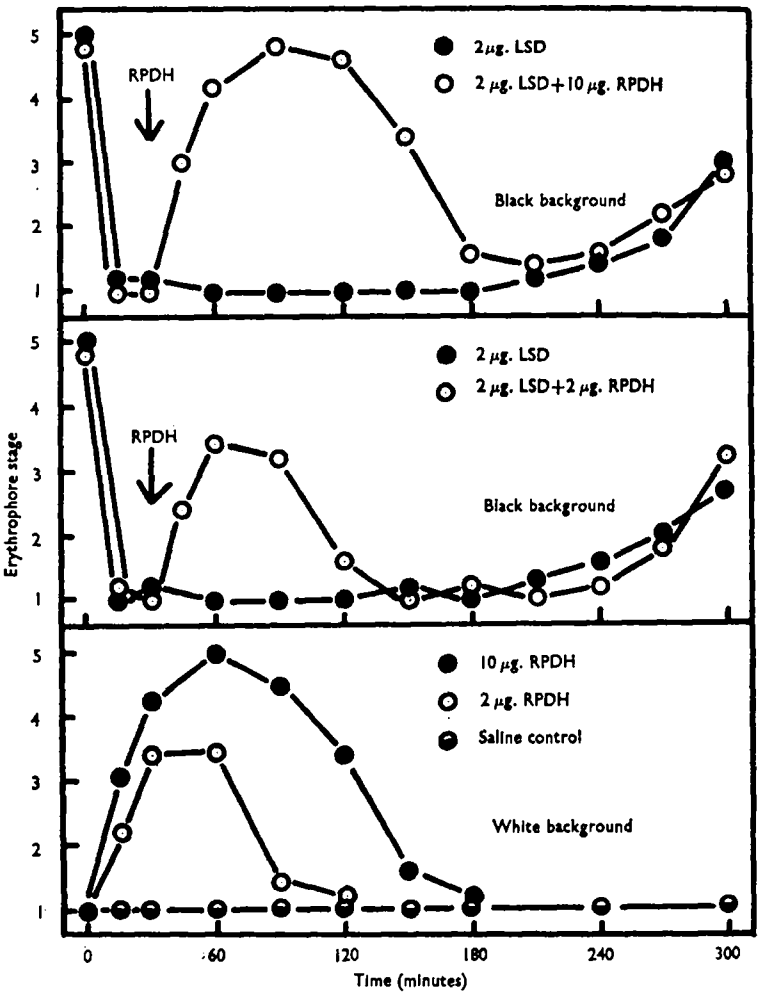

FIG. 2.—Effects of injection of red pigment dispersing hormone (RPDH) subsequent to the administration of LSD into intact *Uca pugilator* adapted to a black background and into intact crabs adapted to a white background. Each arrow indicates the time at which the RPDH was injected.

INJECTION OF RPDH INTO LSD-TREATED INTACT CRABS

This series of experiments was designed to explore the mechanism of action of LSD. Two μg. LSD were injected into each of 20 crabs adapted to a black background. The 20 crabs were then divided into 4 groups of 5 crabs each. Each crab in one group was injected with 5 μg. RPDH per crab 30 minutes after administration of the LSD, whereas each crab in another group received 2 μg. RPDH per crab. The crabs in the remaining 2 groups then received saline. For comparative purposes corresponding dosages of RPDH were assayed on intact crabs adapted to a white background. The experiment was repeated once and the averaged data are shown in *Fig.* 2. It is clear that the crabs

whose red pigment had become concentrated as a result of the LSD treatment showed approximately as large a response to the injected RPDH as did the crabs whose red pigment had initially become concentrated as a result of having been kept on a white background. It can be readily seen that, although LSD had no effect on the response evoked by RPDH, it was able to antagonize partially the action of 5-HT.

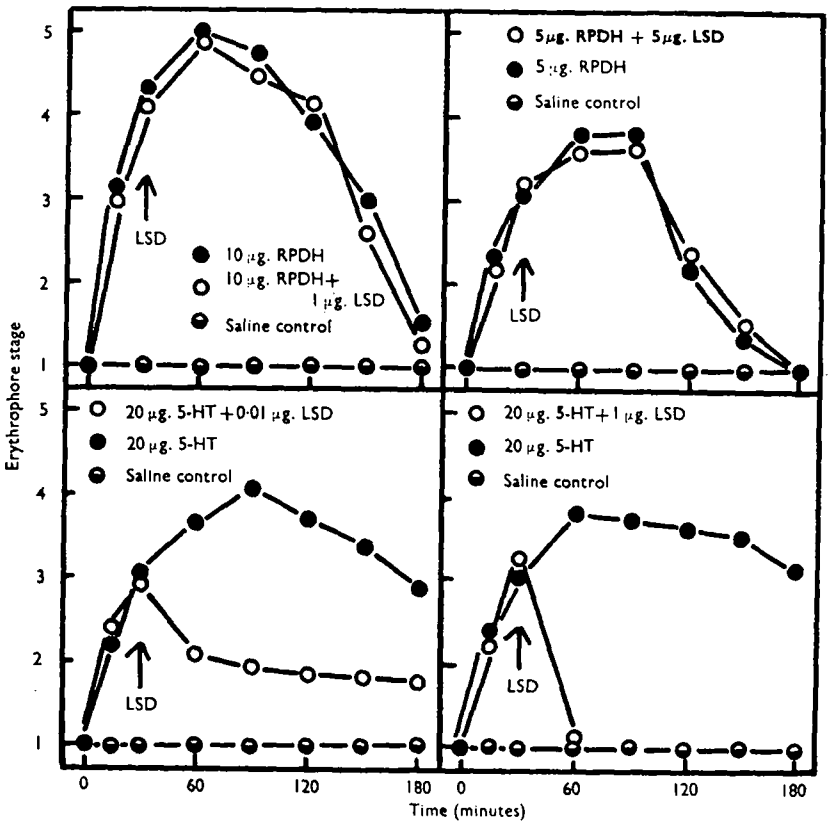

FIG. 3.—Effects of injection of LSD subsequent to the injection of either red pigment dispersing hormone (RPDH) or 5-hydroxytryptamine (5-HT) into intact *Uca pugilator* adapted to a white background. Each arrow indicates the time at which LSD was injected.

INJECTION OF LSD INTO INTACT CRABS SUBSEQUENT TO THE ADMINISTRATION OF RPDH AND 5-HT

In this experiment red pigment dispersion was first evoked by injecting either RPDH or 5-HT into intact crabs adapted to a white background. Thirty minutes later a given dosage of LSD was administered. The averaged results are shown in *Fig.* 3 where each curve is based on data from 10 crabs.

ASSAY OF BLOOD FROM LSD-TREATED CRABS FOR CHROMATOPHOROTROPIC ACTIVITY

Intact crabs adapted to a black background were each injected with 2 μg. LSD. Fifteen minutes later a total of 0·6 ml. blood was withdrawn from 4–5 crabs with a syringe and a hypodermic needle. This blood was immediately injected in a dose of 0·1 ml. per crab into each of 6 eyestalkless crabs of the light patch variant which had fully dispersed red pigment (Rao and Fingerman, 1968). Blood withdrawn from untreated crabs adapted to a black background for at least 2 hours was injected into another group of 6

eyestalkless crabs. A third group of 6 eyestalkless crabs received blood from intact crabs that had adapted to a white background, and the final group of 6 eyestalkless crabs received an injection of 1 μg. LSD. The experiment was repeated once and the averaged data are given in *Fig.* 4. The blood of crabs from a white background evoked a greater pigment-concentrating response than did the blood of the crabs from a black background. In contrast to the situation in intact crabs, LSD itself failed to evoke red pigment concentration in eyestalkless *Uca*. However, the blood from the LSD-treated intact crabs was able to elicit a pronounced red pigment concentration. In fact, the red pigment concentrating potency of the blood from LSD-treated crabs adapted to a black background was significantly higher ($P < 0.05$) than that of blood from intact crabs adapted to a white background. This red pigment concentrating response caused by the blood of the LSD-pretreated crabs could not have been due to any LSD that might still have been in the blood because eyestalkless crabs will not respond to LSD, but must have been due to a change in the relative titres of RPCH and RPDH in the blood. Possible reasons for this change will be discussed below.

Assay of LSD and an Acetone-soluble Fraction of the Eyestalk on Erythrophores *in vitro*

To establish beyond doubt that LSD has no direct effect on the erythrophores of this crab, assays of this substance were also performed on isolated legs. The results are shown in *Fig.* 5, in which each point is based on results from a total of 18 legs. It is clear that LSD does not evoke red pigment concentration *in vitro*. However, the erythrophores are indeed responsive *in vitro* to RPCH from the eyestalk (*Fig.* 5).

DISCUSSION

Our observation that LSD evokes a colour change in intact but not eyestalkless specimens of *Uca pugilator* is in agreement with the findings of Aoto (1963) with *Palaemon paucidens*. However, LSD evokes red pigment concentration in eyestalkless *Palaemon squilla* (Östlund and Fänge, 1956). We have shown here for the first time that LSD evokes only red pigment concentration in *Uca*. But LSD evoked both pigment dispersion and concentration in the red and the white chromatophores of *Palaemon paucidens*, depending upon whether the pigment was initially concentrated or dispersed (Aoto, 1963). It is of interest to note that LSD seems to have a

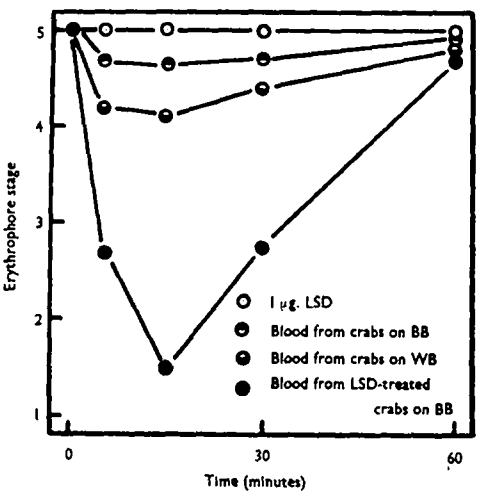

FIG. 4.—Effects of LSD and of blood withdrawn from LSD-treated and uninjected intact *Uca pugilator* adapted to black (BB) or white (WB) backgrounds on the erythrophores of eyestalkless crabs with maximally dispersed red pigment.

greater pigment dispersing effect than pigment concentrating effect in intact *Palaemon paucidens*. Furthermore, in this prawn 5-HT also evokes pigment dispersion in both the red and the white chromatophores. But in *Palaemon squilla* (Östlund and Fänge, 1956) and in *Uca pugilator* (*Figs.* 1, 3) 5-HT and LSD evoke opposite responses; the former caused red pigment dispersion whereas LSD evoked red pigment concentration. Whether or not any antagonism or synergism exists in the actions of 5-HT and LSD on the chromatophores of *Palaemon squilla* and *Palaemon paucidens* has not been determined. In several systems LSD and its derivatives show anti-5-HT activity (Goodman and Gillman, 1966). We have shown earlier (Rao and Fingerman, 1970) that *in vitro* 5-HT does not

activate the erythrophores of *Uca pugilator*. The red pigment dispersing effect of 5-HT *in vivo* is mediated by the neuroendocrine system. The present experiments have provided new information on the mechanism of action of LSD on crustaceans with respect to colour changes. LSD had no effect on the erythrophores either in eyestalkless crabs (*Fig.* 4) or *in vitro* (*Fig.* 5), which clearly shows that the colour change resulting from the administration of LSD is due to an indirect effect of the drug, the LSD presumably affecting the neuroendocrine system. It was shown earlier (Rao and Fingerman, 1970) that 5-HT stimulates the release of RPDH and it is through this indirect mechanism that 5-HT induces the red pigment dispersing response in *Uca pugilator*. The fact that LSD antagonized the action of 5-HT but not that of injected RPDH (*Fig.* 3) strongly suggests that LSD exerts its effect by inhibiting the release of RPDH from the neuroendocrine system. We have also considered the alternative possibility that LSD may stimulate the release of RPCH. If LSD were to stimulate the release of RPCH, it might be expected that the relative effectiveness of the injected RPDH would decrease because Brown (1950) has shown that RPDH and RPCH in *Uca* are mutually antagonistic; the presence of a large quantity of one of these hormones suppresses the effect of the other. Because LSD failed to counteract the effect of injected RPDH it would appear that the LSD-treated crabs from the black background had no more RPCH in their blood than did the control crabs which were on a white background where the red pigment became concentrated normally. Then how do we explain the seemingly high RPCH titres in the blood of intact crabs on a black background which received an injection of LSD (*Fig.* 4)? Evidence from other crustaceans, for example the crayfish *Cambarellus shufeldti* (Fingerman, 1957), indicates that RPDH and RPCH are constantly being released, the relative quantities depending on the physiological state of the organism. The finding that intact crabs whose erythrophoric pigment is dispersed as a consequence of their being adapted to a black background have RPCH in their circulating blood (*Fig.* 4) corroborates the above observation. If LSD can inhibit the release of RPDH, then the relative effectiveness of the circulating RPCH would increase greatly because of the absence

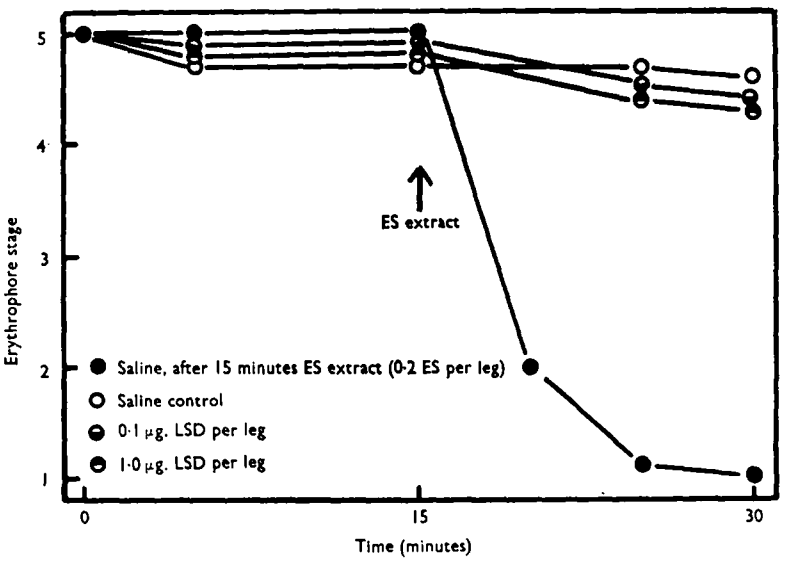

FIG. 5.—Effects of LSD and the acetone-soluble fraction of the eyestalk (ES) on erythrophores in legs isolated from intact *Uca pugilator* adapted to a black background.

of the antagonistic RPDH in the blood and would account for the relatively high red pigment concentrating potency of the blood from the LSD-treated crabs. It is of interest to recall that this relatively high red pigment concentrating potency of blood was not apparent in the *in vivo* injections of RPDH (*Fig.* 2). The injected RPDH evoked as much response in intact crabs on a black background whose red pigment had become concentrated as a result of the LSD treatment as in intact crabs adapted to a white background. As discussed earlier, it is apparent that the relative potency of RPCH in the blood of these crabs is approximately the same. It is reasonable then to assume that the intact crabs adapted to a white background secreted excess RPCH to counteract the injected RPDH. The resulting increase in the effective titre of RPCH in the crabs on a white background appears to be equal to that resulting from LSD treatment (presumably the result of inhibition of release of the antagonistic RPDH) of intact crabs on a black background, so that the response of erythrophores to a given quantity of RPDH is the same in these two types of animals. In view of these considerations, it seems more likely that LSD exerts its action by inhibiting the release of RPDH rather than by stimulating the release of RPCH. However, until refined techniques are developed to determine precisely the titres of these hormones in blood, it is difficult to rule out the possibility that LSD stimulates the release of RPCH.

The mechanism of release of crustacean chromatophorotropins is uncertain. The results of this study and the finding of 5-HT in the central nervous system of *Uca pugilator* (Spirtes and Fingerman, 1969) strongly favour further investigations of the implications of biogenic amines in the control of release of chromatophorotropins in *Uca*. Similar investigations with other crustaceans and efforts to localize the hormones and amines in the nervous system should prove fruitful.

In *Xenopus laevis* it is clear that LSD evokes melanin concentration indirectly by inhibiting the release of intermedin from the pituitary gland (Burgers and Imai, 1962) whereas 5-HT evokes melanin dispersion by acting directly on the melanophores (Van de Veerdonk, Huismans, and Addink, 1961). However, in *Uca pugilator* the effects of both LSD (*Fig.* 5) and 5-HT (Rao and Fingerman, 1970) on the erythrophores are indirect. But the guppy, *Lebistes* (*Poecilia*) *reticulatus*, responds to LSD by exhibiting melanin dispersing reactions both *in vivo* and *in vitro* (Cerletti and Berde, 1955; Berde and Cerletti, 1956). This *in vitro* response of the melanophores in the guppy to LSD may be due to the fact that melanophores of teleosts are innervated (the LSD stimulating the nerve, the chromatophore directly, or both) but there is no evidence that chromatophores of amphibians and crustaceans are innervated (Prosser and Brown, 1961).

SUMMARY

1. A black background fosters maximal dispersion of the pigment in the erythrophores of intact fiddler crabs, *Uca pugilator*, whereas a white background promotes maximal concentration of this pigment. Lysergic acid diethylamine (LSD) evoked marked concentration of the pigment in the erythrophores of intact crabs kept on a black background but had no effect on the erythrophores of intact crabs maintained on a white background. Furthermore, the erythrophores with maximally dispersed pigment in eyestalkless crabs or in legs isolated from intact crabs after adaptation to a black background were also unaffected by LSD.

2. RPDH (red pigment dispersing hormone) evoked a response in LSD-treated intact crabs on a black background and in intact crabs adapted to a white background.

3. RPDH is known to stimulate the erythrophores directly whereas the red pigment dispersing effect of 5-HT (5-hydroxytryptamine, serotonin) is an indirect one mediated by the neuroendocrine system. Although LSD failed to antagonize the action of RPDH it inhibited the red pigment dispersing action of 5-HT.

4. This study reveals that the colour change reaction resulting from LSD administration also is mediated by the neuroendocrine system of the crab. LSD most likely

acts by inhibiting the release of RPDH although stimulation of release of RPCH (red pigment concentrating hormone) can not be completely ruled out at this time.

5. The blood of LSD-pretreated crabs was able to evoke red pigment concentration in eyestalkless *Uca*, although LSD itself had no effect. The implications of this finding are discussed in the light of the well established mutual antagonism between RPDH and RPCH.

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Key Word Index: Biogenic amines, chromatophores, chromatophorotropin, colour change, Crustacea, erythrophores, fiddler crab, 5-hydroxytryptamine, lysergic acid diethylamide, *Uca pugilator*.