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Relationship between the Response of Melanophores in the Fiddler Crab, *Uca pugilator*, and the Concentration of Eyestalk Extract*

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Summary. 1. Eyestalks from the fiddler crab, *Uca pugilator*, were extracted directly in either water or absolute ethyl alcohol. Dosage-response curves for these extracts were then determined.

2. The melanin-dispersing response produced by the alcohol soluble material increased sharply with increasing concentration of the extract between 1 and 80 eyestalks/ml. In contrast, the dosage-response curves for the extracts prepared directly in water did not rise at all as sharply as did that of the alcohol extract.

3. A water extract seems to contain a substance which at the higher concentrations antagonized the melanin-dispersing hormone. Elimination of this antagonist by the technique of gel filtration resulted in an increased melanin-dispersing activity.

4. The possible action of absolute ethyl alcohol which results in a preparation of melanin-dispersing hormone from the eyestalk that is much more active at higher concentrations than a similar extract prepared in water is discussed.

Introduction

Sandeen (1950) reported that the melanophores of *Uca* appear to be capable of increased response with increasing concentration of hormone only up to a certain point and any concentration above that elicits no greater response. She suggested that there may be a physiological limit to the response which renders it impossible to demonstrate high quantities of hormone in an extract. Kleinholz and Kimball (1965) tested different concentrations of eyestalks from *Pandalus* for melanin-dispersing activity on *Uca pugilator* and found that the upper threshold concentration is about 0.2 eyestalk/ml with their method of assay. The aforementioned investigators used extracts of eyestalks prepared directly in an aqueous medium.

Recently one of us (Rao, 1967) found that it is possible to demonstrate much higher titers of melanin-dispersing hormone if it is extracted from the eyestalks with absolute ethyl alcohol instead of water. The alcohol soluble hormone from the optic ganglia of the crab, *Ocypode macrocera*, when prepared in a concentration of one eyestalk per dose

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produced a melanin-dispersing response with the assay method of Sandeen that was 18 times more than that of an extract of the same concentration prepared in physiological saline. In view of this finding we felt it would be worthwhile to determine whether there is indeed a physiological limit of response to melanin-dispersing hormone in Uca pugilator.

Material and Methods

Specimens of Uca pugilator collected in Panacea, Florida, and shipped to New Orleans were used in the present study. In the laboratory the crabs were maintained in aquaria containing artificial sea water. Eyestalkless crabs with maximally concentrated melanin were used in the assays for melanin-dispersing hormone. The eyestalk extracts were prepared according to the following three methods.

Method I. Eighty fresh eyestalks were triturated with a glass rod in an embryological watch glass and extracted with 1.0 ml of crustacean saline (Pantin, 1934). The extract was then centrifuged at 1500×g for 10 minutes. From this stock solution a series of 8 concentrations ranging from 0.1 to 80 eyestalks/ml was prepared using the saline as a diluent.

Method II. Two hundred fresh eyestalks were homogenized in 10.0 ml of distilled water and centrifuged at 17,300×g for 15 minutes. The supernate was freeze-dried and the residue was dissolved in 1.0 ml of distilled water and again centrifuged at 17,300×g for 15 minutes. To the supernate was then added an equal volume of 200% saline. The resulting extract contained the equivalent of 100 eyestalks/ml. From this stock solution a series of 8 concentrations ranging from 0.1 to 100 eyestalks/ml was prepared.

Method III. Eighty fresh eyestalks were triturated in an embryological watch glass with a glass rod and extracted with 10.0 ml of absolute ethyl alcohol. The extract was then centrifuged for 10 minutes at 1500×g. The supernate was decanted into a porcelain dish and allowed to evaporate overnight at room temperature (24—26°C). The residue was then extracted with 1.0 ml of the physiological saline. This stock solution has a concentration of 80 eyestalks/ml. A series of 8 concentrations ranging from 0.1 to 80 eyestalks/ml was prepared using this stock solution also.

Each of the above extracts was injected into eyestalkless Uca pugilator in a dose of 0.05 ml/crab. The chromatophores were staged using the system of Hoaglen and Sloane (1931). The integrated melanin-dispersing response was calculated according to the method of Sandeen (1950). According to her scheme the sum of the mean chromatophore stages for all the periods of observation for the duration of the response is subtracted from the sum for the control group of crabs. The melanophores were staged at the time of injection of the extract and 15, 30 and every 30 minutes thereafter until the effect of the extract wore off. The dosage-response curves presented herein are based on 10 injected test animals for each concentration of extract. The extracts prepared by Methods I and II will be referred to below for the sake of simplicity as the “water extracts.”

Results and Discussion

The dosage-response curves for the three extracts are shown in Fig. 1. The noteworthy feature of these results is how strikingly different