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Antagonism of the Inhibitory Effect of the Polychlorinated Biphenyl Preparation, Aroclor 1242, on Color Changes of the Fiddler Crab, *Uca pugilator*, by Norepinephrine and Drugs Affecting Noradrenergic Neurotransmission

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Polychlorinated biphenyls (PCBs) have become globally distributed pollutants. These substances have not only been found to inhibit molting (FINGERMAN & FINGERMAN 1977) and limb regeneration (FINGERMAN & FINGERMAN 1979) in the fiddler crab, Uca pugilator, but also to affect the color changes of this crab (FINGERMAN & FINGERMAN 1978). The pigment in the melanophores of Uca pugilator kept in white containers and exposed to the PCB preparation, Aroclor 1242, became less dispersed than in untreated crabs. Furthermore, the eyestalks of Aroclor 1242-exposed crabs contained more melanin-dispersing hormone (MDH) than did those of control crabs. These results suggested that Aroclor 1242 was not affecting the synthesis of this neurohormone, but rather its release. Support for this hypothesis was the observation that the medulla terminalis X-organ in the eyestalks of Uca pugilator exposed to Aroclor 1242 contained more stainable neurosecretory material than in control crabs (NAGABHUSHANAM et al. 1979). Presumably, at least part of this stainable material was MDH. More recently, evidence has been obtained that norepinephrine (NE) triggers release of MDH in this species of fiddler crab (HANUMANTE et al. 1980, FINGERMAN et al. 1981). In view of the possible role of NE in triggering MDH release in Uca pugilator, experiments were conducted to determine whether Aroclor 1242 might be exerting its effect of preventing the release of MDH in fiddler crabs kept in white containers by affecting noradrenergic neurotransmission. We also wished to determine whether the melanin of Aroclor 1242-exposed crabs kept in black pans will become less dispersed than in control crabs, as occurs with Aroclor 1242-exposed crabs kept in white pans.

MATERIALS AND METHODS

Adult male fiddler crabs, Uca pugilator, from the area of Panacea, FL, were used. The crabs were exposed to an illumination of 1190 lux and a temperature of 24°C during the experiments.

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The melanophores were staged using the system of HOGBEN & SLOME (1931). According to their system, stage 1 represents maximal pigment concentration, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. The melanophores staged were those seen through the cuticle on the anteroventral surface of the second walking leg on the right side.

Norepinephrine hydrochloride (Sigma), clonidine hydrochloride (Boehringer-Ingelheim), mianserin hydrochloride (Organon), and nisoxetine hydrochloride (Lilly) were used. These compounds were prepared in a concentration of 20 µg of the free compound per dose in crustacean physiological saline (PANTIN 1934). The volume injected into each crab was 0.05 ml. The controls received saline alone. Each substance was tested on a total of 10 crabs. The means ± the SEM were determined.

The experimental crabs were immersed in a solution of Aroclor 1242 (Monsanto Lot Number G266K). It was first dissolved in acetone (8 mg/ml) and then diluted 1000-fold in artificial sea water (Instant Ocean, Aquarium Systems) for use. Control crabs were exposed to artificial sea water containing 0.1% acetone, the same concentration as in the diluted solution of the Aroclor 1242. The solutions in which the crabs were immersed were changed daily.

RESULTS

Crabs were taken from the stock supply, placed at noon into white enameled pans containing artificial sea water, and exposed to an illumination of 1190 lux. Throughout this experiment the crabs were kept in white pans. Twenty-four hours later two groups of crabs were selected from those in the white containers such that the mean melanophore stage of each group was 2.5 ± 0.1. One group consisting of 84 crabs served as the experimental group and the second, 87 crabs, served as the controls. The experimental crabs were put in sea water containing acetone and Aroclor 1242 whereas the control crabs were in sea water that contained acetone alone. Forty-eight hours later the mean stage of both groups of crabs was again determined. The mean of the control group was 2.7 ± 0.1 whereas the mean melanophore stage of the Aroclor 1242-exposed crabs was less than for the controls, only 1.6 ± 0.1. With crabs in white pans, the Aroclor 1242, as expected, had prevented the melanin from becoming as dispersed as it was in the control group. The circadian rhythm (ABRAMOWITZ 1937) of pigment dispersion of Uca pugilator fosters increased melanin dispersion during the daytime. The slight increase in the mean of the control group, 0.2 of a stage, that occurred during the 48 hours of exposure, was probably due to a direct response of the melanophores to the illumination (BROWN & SANDEEN 1948), bright light favoring pigment dispersion, and the circatidal rhythm of color change of fiddler crabs (BROWN et al. 1953).

Sixty crabs, six groups of 10 each, were then selected at random from the Aroclor 1242-exposed crabs. These 60 crabs continued to be exposed to Aroclor 1242. Also, one group of 10 crabs

was selected at random from the acetone-sea water control crabs. These 10 crabs continued to be kept in acetone sea water. All seven groups were still kept in white containers. One group of the Aroclor 1242-exposed crabs and the 10 crabs selected from the original control group received an injection of physiological saline alone. The remaining groups of Aroclor 1242-exposed crabs received an injection of nisoxetine, mianserin, NE, clonidine, or NE plus clonidine. The melanophores of all the crabs were staged at the time of injection and 15, 30, 60, 90, and 120 minutes thereafter. The results are presented in Table 1.

TABLE 1

Mean Melanophore Stages ± SEM of the Control and Experimental Crabs that Were Kept on a White Background

Treatment	Minutes					
	0	15	30	60	90	120
0.1% Acetone Sea Water + Saline	2.5±0.1	2.1±0.2	2.0±0.2	2.0±0.2	2.1±0.2	1.9±0.2
Aroclor 1242 + Saline	1.6±0.1	1.6±0.1	1.7±0.1	1.7±0.1	1.6±0.1	1.6±0.1
Aroclor 1242 + Nisoxetine	1.2±0.1	1.8±0.2	1.7±0.2	1.7±0.3	1.9±0.3	2.0±0.3
Aroclor 1242 + Mianserin	1.5±0.2	2.0±0.2	2.1±0.3	2.2±0.3	2.4±0.3	2.4±0.3
Aroclor 1242 + Norepinephrine	1.7±0.2	3.1±0.2	3.1±0.3	2.8±0.3	2.6±0.3	2.7±0.3
Aroclor 1242 + Clonidine	1.6±0.3	2.6±0.3	2.4±0.4	2.4±0.4	2.4±0.4	2.2±0.3
Aroclor 1242 + Norepinephrine + Clonidine	1.9±0.2	3.2±0.2	3.9±0.2	3.4±0.2	2.8±0.1	2.9±0.1

Injection of saline into the crabs in the acetone sea water has a melanin-concentrating effect, but saline injection into the Aroclor 1242-exposed crabs produced no significant response to the saline. The difference between the responses was probably due to the fact that the melanin was originally more dispersed in the acetone sea water crabs than in the Aroclor 1242-exposed crabs and the dilution of the MDH in the blood by the injected saline had a greater effect on those crabs whose melanin was more dispersed at the time of injection, resulting in melanin aggregation occurring in the saline-injected group which was darker originally.

All of the drugs, unlike the saline, evoked a melanin-dispersing response. The crabs that received NE + clonidine exhibited the largest response. The clonidine and NE were synergistic, the combination producing a larger response than either did alone.

The experiment described above, was in agreement with the earlier observations (FINGERMAN & FINGERMAN 1978) that the melanin of Aroclor 1242-exposed crabs kept on a white background becomes less dispersed than in control crabs. The next experiment performed was designed to determine whether the melanin of crabs transferred from a white to a black container at the time of initial exposure to Aroclor 1242 would also become less dispersed than in control crabs. A black background fosters melanin dispersion (BROWN & HINES 1952) as does the circadian rhythm of color change during its day phase. Crabs were again selected from the stock supply, placed at noon in white pans containing artificial sea water alone and exposed to an illumination of 1190 lux. The following noon 28 control and 28 experimental crabs were selected. The mean melanophore stage of the control group was 2.5 ± 0.1, that of the experimental group was 2.4 ± 0.1. All of these crabs were then transferred from white to black containers. The black pans into which the control crabs were placed held acetone sea water whereas the black pans into which the experimental crabs were placed contained acetone sea water plus Aroclor 1242. The melanophore stage of each crab was then determined at noon for the next four days (Table 2). The melanin of the acetone sea water crabs in the black pans became, as expected, more dispersed than it had been at the outset. However, surprisingly, there was no significant difference between the mean melanophore stages of the control and experimental crabs kept in the black containers, the melanin of these Aroclor 1242-exposed crabs becoming essentially just as dispersed as that of the control crabs in the acetone sea water.

TABLE 2

Mean Melanophore Stages ± SEM of Control and Experimental Crabs Transferred from a White to a Black Container

Treatment	Days				
	0	1	2	3	4
0.1% Acetone Sea Water	2.5±0.1	3.9±0.1	4.1±0.1	4.1±0.1	4.3±0.2
Aroclor 1242	2.4±0.1	3.8±0.2	4.1±0.1	4.3±0.1	4.1±0.2

DISCUSSION

The results shown in Table 1 are consistent with the hypothesis of HANUMANTE et al. (1980) and FINGERMAN et al. (1981) that NE produces melanin dispersion in the fiddler crab by triggering MDH release. Furthermore, the known actions of the drugs used herein,

as will be discussed below, suggest that Aroclor 1242 prevents melanin dispersion in crabs in white containers by reducing the amount of NE available at appropriate synapses to trigger release of sufficient MDH. The circadian rhythm of color change of these crabs would be fostering melanin dispersion during the daytime, when the readings reported herein were taken. Consequently, in accordance with the hypothesis of HANUMANTE et al. (1980) and FINGERMAN et al. (1981) during the daytime impulses would be generated to cause release of NE which would trigger MDH release. However, Aroclor 1242 would be inhibiting release of sufficient NE in crabs that were in white containers; as a result less pigment dispersion would occur in the integument of these Aroclor 1242-exposed crabs than in the controls.

NE is known to stimulate mainly alpha-adrenoceptors (MAXWELL 1971, JENKINSON & BENTLEY 1971). Clonidine is both a presynaptic and postsynaptic alpha-adrenoceptor stimulator (ANDÉN et al. 1970, McLENNAN & BENTLEY 1971). Presynaptic alpha-adrenoceptors function in a local negative feedback control of NE release (LANGER 1977, STARKE 1977). The result is that agonists of these presynaptic receptors inhibit NE release whereas antagonists of these receptors enhance NE release. Clonidine, being a stimulator of both presynaptic and postsynaptic alpha-adrenoceptors could have produced a mixed response. However, the clear melanin-dispersing response that it produced shows that at least when the circadian rhythm is calling for melanin dispersion, presumably through the MDH-releasing action of NE, the postsynaptic NE-mimicking action of clonidine is the dominant one. Mianserin is a presynaptic alpha-adrenoceptor blocker (BROGDEN et al. 1978) and also inhibits NE reuptake (GOODLET et al. 1977, DOXEY et al. 1978). This action of mianserin would, therefore, potentiate the action of NE when the circadian rhythm calls for melanin dispersion, resulting in increased dispersion of this pigment in accordance with the hypothesis that NE triggers MDH release. Nisoxetine, like mianserin, also inhibits NE reuptake (KOE 1976), but nisoxetine does not block presynaptic alpha-adrenoceptors. The fact that nisoxetine produced melanin dispersion is in keeping with the action of this drug of inhibiting NE reuptake.

The fact that NE, clonidine, and NE + clonidine produced melanin dispersion in Aroclor 1242-exposed crabs on a white background to at least the level (stage 2.5) seen at zero time in the acetone sea water controls on a white background (Table 1) shows that Aroclor 1242 did not prevent melanin dispersion by blocking postsynaptic alpha-adrenoceptors. Furthermore, the fact that Aroclor 1242-exposed crabs on a black background that fosters melanin dispersion could cause their melanin to become not only essentially just as dispersed as the melanin in control crabs on a black background but also even more dispersed (Table 2) than occurred in the control crabs in the white container (Table 1) argues against hypotheses that Aroclor 1242 prevents melanin dispersion by inhibiting NE synthesis or augmenting NE breakdown. The combination of melanin-dispersing tendencies fostered by a black background and the circadian rhythm of melanin dispersion

was strong enough to override the inhibitory effect of Aroclor 1242 on melanin dispersion that is apparent in Aroclor 1242-treated crabs kept on a white background. Consequently, we are left with the hypothesis proposed above, namely that Aroclor 1242 prevents melanin dispersion by reducing the amount of NE available at appropriate synapses below that which is sufficient to trigger release of an appropriate amount of MDH. Perhaps Aroclor 1242 is accomplishing this by stimulating presynaptic alpha-adrenoceptors and/or by increasing the rate of NE reuptake into presynaptic neurons.

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