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Exposure to the Water Soluble Fraction of Crude Oil or to Naphthalenes Alters Breathing Rates in Gulf Killifish, *Fundulus grandis*

Lisa C. Russell and Milton Fingerman

Department of Biology, Tulane University, New Orleans, LA 70118

Alteration in breathing rate has been used to monitor the effects of pollutants on fishes. Particularly pertinent to the study described herein are the observations of Rice et al. (1977) that the water soluble fractions (WSF) from Cook Inlet crude oil, Prudhoe Bay crude oil and No. 2 fuel oil increased the breathing rate of pink salmon, Oncorhynchus gorbuscha, fry. However, possible underlying neurological mechanisms for this response have not been identified. Pollutant-induced changes in a fish's breathing rate may indicate neurochemical imbalances in the brain.

Exposure of the longnose killifish, Fundulus similis, to the WSF of petroleum resulted in accumulation of naphthalenes from this WSF in high levels in the brain (Dixit and Anderson 1977). Russell (1980), using the Gulf killifish, Fundulus grandis, found that naphthalenes become localized mainly in the prosencephalon and medulla oblongata. In fishes, the central nervous control site of breathing is located in the medulla oblongata, which is also a primary site in vertebrates of the neurotransmitter, dopamine. Various organic compounds have been found to ultimately produce reductions in the whole brain concentration of dopamine in fishes. This was found for Fundulus grandis by Fingerman and Russell (1980) with the polychlorinated biphenyl, Aroclor 1242; for the mullet, Mugil cephalus, by Thomas et al. (1981) with dibenzofuran; and for the channel catfish, Ictalurus punctatus, by Fingerman and Short (1983) with naphthalene. In view of these effects of various pollutants on breathing rate and the brain dopamine level in fishes, experiments were performed to determine the effects of (a) the WSF of South Louisiana crude oil, (b) two of its most toxic components (naphthalene and 2,6-dimethylnaphthalene) and (c) the dopamine precursor, L-DOPA, on the breathing rate of Fundulus grandis. These experiments would not only reveal whether the WSF and naphthalenes affect the breathing rate but also whether it might be affected by the dopamine concentration in the fish.

MATERIALS AND METHODS

Adult female Fundulus grandis obtained from the marshes around Hopedale and Shell Beach, Louisiana, were donated by the owner

of Pip's, a local bait shop. The individuals had a weight range of 11-28 g and a total length of 9.0-16.7 cm. The stock fish were maintained in aerated, filtered 50% sea water (Instant Ocean, Aquarium Systems). A lighting regimen of LD 12:12 with the lights on from 0600 to 1800 hr daily was maintained by the use of an electric timer. The intensity of illumination at the water surface was 1120 lx. Water temperature was kept at 25°C. Stock fish were fed commercial fish food daily. But fish being used in an experiment were not fed.

The WSF of South Louisiana crude oil (Shell Oil Company) was prepared by the method of Anderson et al. (1974), and had a concentration of 500 ppm. The solutions of naphthalene (Matheson Coleman and Bell) and 2,6-dimethylnaphthalene (Pfaltz and Bauer) were prepared by dissolving 8 mg of each in 10 ml acetone (Laughlin and Neff 1979), then diluting an aliquot with 50% sea water. The final acetone concentration was 625 ppm, and the final naphthalene and 2,6-dimethylnaphthalene concentrations were 0.5 ppm. L-DOPA (Sigma) was dissolved in fish saline (Dreyer 1930) in a concentration of 1 mg/ml. The injected dose was 0.1 ml. Thus, each fish received 100 µg L-DOPA, which was used in these experiments instead of dopamine because L-DOPA crosses the blood-brain barrier more easily.

When fish were exposed to the WSF, the controls were kept in 50% sea water. Controls for naphthalene and 2,6-dimethylnaphthalene were exposed to a stock solution of 625 ppm in acetone in 50% sea water. Control fish for the L-DOPA experiments received an injection of 0.1 ml fish saline. The L-DOPA recipients and their corresponding controls were kept in 50% sea water.

To correct for differences in the breathing rate of individual fish, the data (see Tables 1 and 2) are presented as percents based on the opercular counts recorded at predetermined times for each fish before (on Day 1) and after (on Days 2 and 3) the fish were exposed to a pollutant or injected with L-DOPA. For example, the number of opercular movements of each fish counted at 1100 hr on Day 2 was divided by the number of that fish's opercular movements counted at 1100 hr on Day 1. This dividend was multiplied by 100 to yield the percent. Each experiment was performed twice with 10 experimental and 10 control fish each time the experiment was performed. The data from both experiments were qualitatively the same. Each percent value in the tables thus represents the mean for 20 fish. Standard errors of the means (SEM) of the percents were calculated and are shown in Tables 1 and 2.

The first experiments performed were designed to determine whether the WSF, naphthalene or 2,6-dimethylnaphthalene would produce a change in opercular breathing rate. For testing with each substance, fish were selected from the stock supply, placed at 1200 hr on Day 0 in testing bowls containing only 50% sea water, one per bowl, and allowed to adjust to their new surroundings. The following day (Day 1) each fish's opercular movements were

Table 1. Percent change (±SEM) in opercular breathing rate after exposure to WSF, naphthalene, 2,6-dimethylnaphthalene or acetone-50% sea water. The minus signs in front of the percents indicate the rates decreased.

	Hours from start of exposure			
	3	6	9	22.5
WSF-exposed	-15±3	-29±5	-29±3	- 9±2
Controls for WSF-exposed fish (in 50% sea water)	- 9±3	-10±3	-10±3	- 8±2
Naphthalene-exposed	-37±3	-29±3	N.D.*	-26±4
2,6-Dimethylnaphthalene-exposed	-37±4	-24±4	N.D.*	-15±3
Controls for naphthalene-exposed and 2,6-dimethyl-naphthalene-exposed fish (in acetone-50% sea water)	- 6±2	- 3±1	N.D.*	- 5±1

*N.D., not determined

Table 2. Percent change (±SEM) in opercular breathing rate after injection of L-DOPA or saline.

	Hours after injection			
	3	6	9	22.5
L-DOPA-injected	+18±6	+24±6	+26±4	+30±6
Controls (saline-injected)	- 6±2	- 7±1	- 6±2	- 5±1

counted for three minutes at 0630, 1400 and 1700 hr with all the fish still in 50% sea water. At 0800 hr on the next day (Day 2) the fish selected for the experimental groups were exposed to either the WSF, naphthalene or 2,6-dimethylnaphthalene, and the remaining groups served as the appropriate controls. Following exposure or dosage, each fish's opercular movements were then counted on Day 2 at 1100 hr (3 hr exposure) and 1400 hr (6 hr exposure) and on Day 3 at 0630 hr (22.5 hr exposure). For the WSF-exposed fish, counts were also made at 1700 hr (9 hr exposure) on Day 2.

RESULTS AND DISCUSSION

The breathing rates of the control fish decreased slightly on

Days 2 and 3 (Table 1). However, the breathing rates of the fish exposed to the WSF and the naphthalenes decreased significantly compared to their controls.

Experiments were then performed to determine whether L-DOPA which is known to produce dopamine increases in vertebrate brains might affect the breathing rate. The breathing rates were determined at the same time intervals as those of the WSF-exposed fish. Although the breathing rate of the control fish decreased (Table 2), L-DOPA caused significant increases in the breathing rate of treated fish.

The present experiments clearly show that naphthalene, 2,6-dimethylnaphthalene and the water soluble fraction of crude oil significantly reduced the breathing rate of Fundulus grandis, whereas the dopamine precursor L-DOPA significantly increased it.

Naphthalenes and methylnaphthalenes, the most toxic components of crude oil (Anderson et al. 1974), accumulate in high levels in the brain of exposed fishes (Dixit and Anderson 1977; Roubal et al. 1977; Collier et al. 1978; Varanasi et al. 1979). Previous experiments (Russell 1980) have shown that naphthalenes from 500 ppm water soluble fraction of crude oil and from solutions of pure naphthalenes accumulate in the forebrain and hindbrain of Fundulus grandis in concentrations up to five times that in the water. This localization is probably due to the proximity of these brain areas to the olfactory epithelium, heart and gills. In unpublished experiments in this laboratory, exposure to naphthalenes or the water soluble fraction of crude oil caused decreases in whole brain dopamine in Fundulus grandis, whereas L-DOPA produced a dopamine increase.

The reductions of breathing rate and brain dopamine level in Fundulus following exposure to naphthalenes or the water soluble fraction of crude oil suggest these physiological alterations are related. The increased breathing rates in response to L-DOPA (Table 2) also indicate that the brain dopamine level may be related to the breathing rate in Fundulus. However, as mentioned above, Rice et al. (1977) observed an increase in the breathing rate of pink salmon fry exposed to the water soluble fractions of three oils, so these responses to pollutants may be age- or species-specific. Concurrent breathing rate and brain part assays for dopamine and a thorough pharmacological study are needed to confirm this suggested relationship. Breathing rate seems to be an indicator of the neurochemical and/or neurophysiological state of fishes.

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