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Alteration of Thermoregulatory Behavior in Fish by 5-Hydroxytryptamine[1]

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FRYER, J. N. AND D. M. OGILVIE. *Alteration of thermoregulatory behaviour in fish by 5-hydroxytryptamine.* PHARMAC. BIOCHEM. BEHAV. 8(2) 129–132, 1978. – Guppies were exposed for 30 min to doses of 5-HT ranging from 20 to 120 mg/l, and then tested in a horizontal temperature gradient. Atlantic salmon injected with 2 μg/g of 5-HT were similarly tested. The guppies exhibited a biphasic relationship between 5-HT dose and mean selected temperature, i.e., the lowest dose significantly decreased the selected temperature, while the highest dose significantly increased it. In the salmon, injection of 5-HT initially decreased the selected temperature by about 4°C. With time, however, the selected temperature gradually increased until by three hours after injection it exceeded the control value by about 1.5°C. This pattern of temperature change is similar to that observed with rabbits subjected to both systemic and central injection of 5-HT.

Temperature selection Teleosts 5-HT

THE FIRST neurochemical model of temperature regulation in mammals was based upon experiments with cats in which intraventricular injection of norepinephrine produced hypothermia, while injection of 5-hydroxytryptamine increased heat production and resulted in a rise in body temperature [5]. Similar results were obtained with dogs [6] and monkeys [17], however, because of contradictory findings with other species, for example rabbits [2] and mice [12], this model does not appear to be applicable to all mammlian species.

In contrast to mammals, poikilothermic animals have either inadequate or nonexistent physiological mechanisms for thermoregulation, and they typically rely on their behaviour to maintain body temperature. Such animals readily exhibit behavioural thermoregulation (temperature selection) when placed in a thermocline. In fish this response is highly labile and may be altered by thermal acclimation [4], nutritional state [14] and exposure to various chemicals [16, 18–20].

The mechanism of temperature selection is poorly understood, however, it appears that fish possess at least some of the basic temperature regulating mechanisms found in homeotherms. Both peripheral and brainstem temperatures appear to be of importance [11], and the hypothalamus has been implicated in both fish [3] and amphibians [15]. Both 5-HT [25] and catecholamines [23] have been found in fish brains, but whether or not these neurochemicals play a role in the behavioural thermoregulatory responses of fishes is not known. This paper describes the results of an investigation to determine if treatment with 5-HT could alter temperature selection in fish.

METHOD

Adult male guppies *(Poecilia reticulata)* obtained from a commercial supplier, and underyearling Atlantic salmon *(Salmo salar)* provided by the Department of the Environment, Cobequid Fish Culture Station (Collingwood, Nova Scotia) were used for these experiments. The fish were held in 15 gal aquaria, and were kept on a 12L:12D photoperiod at 20°C for at least two weeks prior to use. Guppies were fed a prepared diet (Tetramin) each morning, while salmon were fed No. 2 Trout Food (Ralston Purina) each morning and late in the afternoon.

The thermogradient apparatus consisted essentially of an 8-cm-high rectangular enclosure of sheet aluminium cemented to the surface of an aluminium plate measuring 1.7 × 90 × 240 cm. The enclosure was divided into five lanes 11 cm wide and 185 cm long by strips of black Plexiglas 0.3 cm thick and 3.8 cm high. The lanes were filled with water to a depth sufficient for the test fish to just swim clear of the bottom. A highly stable and relatively linear horizontal gradient of water temperature was produced by cooling one end of the plate (which was inserted 30 cm into a refrigerated bath), and applying heat to the opposite end by means of two 1000-W strip heaters.

In order to record the positions of the fish in the gradient, each lane was divided into 20 equal sections by strings placed across the Plexiglas strips. Section numbers (1 to 20) were painted on two strips of plywood which formed an inclined roof over the center lane of the apparatus. This center divider also served to separate the control and experimental animals since some fish occasionally jumped from one lane to another.

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Throughout these studies the experimental fish (usually in groups of five to a lane) were placed in the two lanes on one side of the center divider, while an equivalent number of control fish were placed in the other two lanes on the opposite side of the divider. Control and experimental fish were therefore tested simultaneously to avoid temporal variation in response, and the control and experimental sides of the apparatus were altered from one experiment to another to avoid introducing any bias due to position.

Water temperatures on both the control and the experimental sides of the apparatus were recorded 5 mm above the surface of the aluminium plate with a telethermometer (Y.S.I., Model 44) and a thermistor probe (Type 402). Measurements were made immediately before the introduction of the fish into the apparatus. During experiments with guppies, temperatures were again determined at hourly intervals, however, in the case of tests with salmon, additional measurements could only be made at the end of the experiments since movements by the observer disturbed these fish and obliterated the temperature selection response. This was of little consequence, however, since gradient temperatures typically changed very little during the course of an experiment.

Prior to temperature selection tests guppies were exposed for 30 min to 20, 40, 80 or 120 mg/l of 5-HT (Baker Chemicals, 5-hydroxytryptamine creatinine sulfate). Five fish were placed in a 1000 ml beaker containing 250 ml of gently aerated water at 20°C and the appropriate concentration of 5-HT. At the same time, five control fish were held under similar conditions in water containing no 5-HT. Although this method of administration was admittedly crude, it seemed a worthwhile and simple approach to use as a first approximation. After 30 min the fish were transferred to the thermogradient apparatus in which the responses of 5-HT treated and control fish were observed simultaneously. Each experiment was replicated three to six times.

To achieve a somewhat better technique of administration, the salmon (which were several times larger than the guppies) were injected intraperitoneally with 2 μg/g of 5-HT dissolved in teleost saline. This method was obviously still not equivalent to an intraventricular injection, however, it was hoped that some of the administered 5-HT would nevertheless cross the blood-brain barrier and reach the central nervous system. Control salmon were injected with an equal volume (0.15 ml) of saline only. For each experiment five controls and five 5-HT-injected fish were tested simultaneously in the thermogradient apparatus immediately following injection. This experiment was replicated three times.

Observations were begun 10 min after the fish had been transferred to the temperature gradient. The position of each fish in the apparatus was determined every two min for two hr. One hr later, observations were resumed for a final 60-min period.

RESULTS

Previous experiments have shown that when 20°C-acclimated guppies were placed in our thermogradient device for four hr, their mean selected temperature ranged from about 28 to 30°C [8]. In the present study the mean control value for 105 fish determined periodically for four hr was 27.7°C. An hourly summary of the effects of graded doses of 5-HT on the selected temperature of guppies is shown in Fig. 1 which depicts the difference between the mean

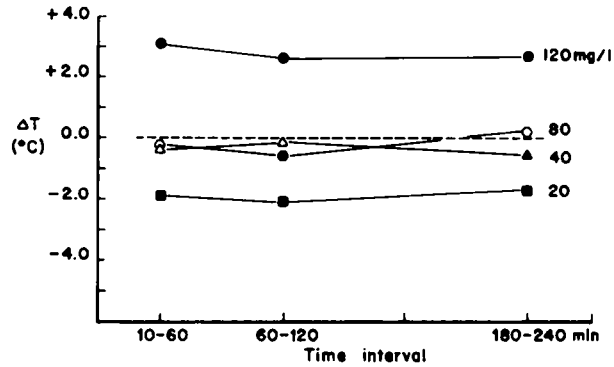

FIG. 1. An hourly summary of the difference (ΔT) between the mean temperatures selected by 5-HT-exposed and control guppies. Each point represents the mean temperature selected by 15 to 40 fish. Closed symbols indicate values significantly different ($p<0.05$) from the controls.

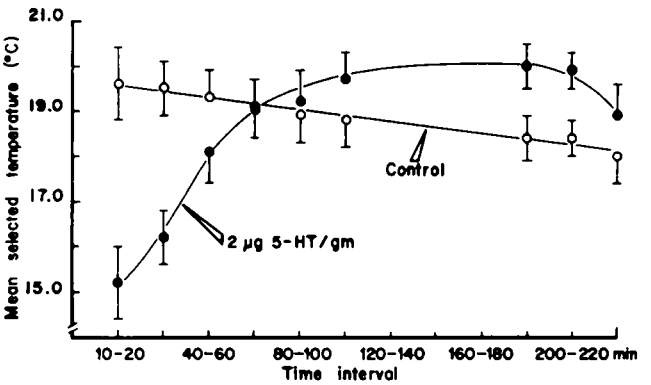

FIG. 2. A summary of mean temperatures selected by 5-HT- (closed circles) and saline-injected (open circles) Atlantic salmon during successive 20-min intervals following injection. Each point represents the mean temperature selected by 15 fish. Vertical bars denote ± 2 SEM.

temperatures selected by 5-HT-treated and control fish. During the 4 hr period following exposure to 5-HT, guppies treated with 20 mg/l selected temperatures which consistently averaged about 2°C lower than those selected by control fish. In contrast to this result, fish exposed to 120 mg/l of 5-HT selected temperatures which remained about 3°C higher than the comparable control values. Intermediate doses of 40 and 80 mg/l had little or no effect on the selected temperature.

A summary of mean temperatures selected by 5-HT-injected and control salmon during successive 20-min intervals is shown in Fig. 2. In the case of the control fish, the mean selected temperature progressively decreased with time such that the value for the final 20-min interval of observation was nearly 2°C lower than the value for the first 10 min of observation. In the case of the 5-HT-injected fish, there was a marked initial depression of the selected temperature to a point which was approximately 4°C lower than the average for the control fish. The mean temperature selected by the 5-HT salmon gradually increased with time, however, and by three hr after injection significantly exceeded the control value by about 1.5°C. By the fourth hour there was no significant difference between the mean temperatures selected by 5-HT-injected and control salmon.

DISCUSSION

In the case of guppies exposed to 5-HT, the observed alterations of selected temperature were complex since a low concentration significantly decreased the selected temperature, while exposure to a high concentration resulted in a significant increase. The reason for this result is not clear, however, a similar biphasic effect on temperature selection has been reported for salmon exposed to graded doses of DDT, a chemical which strongly affects the nervous system [18].

The mean temperature selected by saline-injected salmon progressively decreased during the 4-hr observation period (Fig. 2). Since the temperature initially selected by these fish was approximately 3°C higher than that determined previously for non-injected salmon tested under similar conditions [8], it appears that the trauma of injection may have resulted in a temporary elevation in selected temperature. The observed impairment of thermoregulation in mammals subjected to handling and restraint may represent a comparable phenomenon in mammals, although in this case hypothermia typically is observed [1].

If we assume that the selected temperature of a fish is analogous to the body temperature of a mammal, the time course of alteration in selected temperature observed in the 5-HT salmon is of particular interest since it is similar to the changes in body temperature following injection of 5-HT into rabbits [13]. Not only did such injection cause a fall in body temperature followed by a rise, but in addition, the same result was obtained regardless of whether the 5-HT was administered centrally or systemically. The only difference attributable to the route of administration was that the effective doses were much higher when the drug was administered systematically.

It is of course impossible to say whether or not the 5-HT administered to fish in the present study initiated thermoregulatory responses analogous to those characteristic of mammalian systems. It is of interest, however, that such effects have been previously been reported for poikilotherms. For example, iguanas subjected to bacterial infection developed fever by spending more time at higher environmental temperatures [22]. Moreover, it has been recently reported that fish can also develop a behavioural fever when injected with a pyrogen [21]. There is also a striking parallel between the effects produced by pentobarbital on mammals and fish. This drug is known to affect thermoregulation in mammals [7], and will, for example, greatly accelerate the rate at which the body temperature falls when rats are exposed to cold [24]. In the case of fish, exposure of guppies and salmon to this drug resulted in a significant decrease in selected temperature [9,19]. It therefore appears that agents which alter mammalian temperature regulation may possibly also act on poikilothermic thermoregulation which is largely behavioural.

Many similarities are beginning to emerge in the central control of thermoregulatory responses in both teleosts and mammals. In both groups alterations in behavioural thermoregulation have been produced by manipulation of brainstem temperature [3,7]. In addition, neurons in the fish brain display thermosensitive characteristics similar to those of the mammalian hypothalamus [10]. Perhaps the alterations in the selected temperatures of fish produced by treatment with 5-HT may be a reflection of a common neurochemical link between the thermoregulatory systems of teleosts and mammals.

Because of the method of 5-HT administration in the present study, it is uncertain whether or not any of the amine reached the brain. However, such a possibility should not be discounted. Relatively large doses were used, and it should be recalled that in the rabbit, 5-HT administration produced the same general effect regardless of whether the amine was injected centrally or systematically [13]. Despite obvious limitations, the results of this study are sufficiently encouraging to warrent further investigation which must involve central administration of 5-HT and catecholamines.

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