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FEVER AND THERMAL TOLERANCE IN THE TOAD *BUFO MARINUS*

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Abstract—1 *Bufo marinus* injected with the pyrogen lipopolysaccharide (LPS) exhibited an average behavioural fever of 2 2°C expressed as an increase in mean selected temperature on a thermal gradient Control toads injected with saline did not exhibit an increase in mean selected temperature

2 The thermal tolerance (as measured by the critical thermal maximum, CTM) of pyrogen-treated toads increased significantly over that of saline-injected toads if both groups of toads were incubated at a febrile temperature (32°C) but not if they were incubated at a normothermic temperature (25°C)

3 The importance of permitting ectotherms to become hyperthermic in order to study the physiological correlates of pyrogen treatment is discussed

Key Word Index Fever, behavioural hyperthermia, thermal tolerance, ectotherms, toads

INTRODUCTION

The injection of pyrogenic substances induces behavioural fever in many different ectotherms Behavioural fever is expressed as an increase in the temperature selected by pyrogen-treated individuals Vertebrate ectotherms exhibit behavioural hyperthermia of 2°C or more in response to injection with live or dead bacteria (Vaughn *et al*, 1974; Reynolds *et al*, 1976, Casterlin and Reynolds, 1977; Kluger, 1977) Endogenous pyrogen and prostaglandin E_1, substances associated with a host's immune response, have induced fevers of even greater magnitude (Bernheim and Kluger, 1977; Hutchison and Erskine, 1981) Arthropods exhibit behavioural fevers of 2–10°C in response to treatment with various pyrogenic agents (see Boorstein and Ewald, 1987) Moreover, behavioural fevers confer survival benefits to infected lizards (Kluger *et al*, 1975), fish (Covert and Reynolds, 1977) and grasshoppers (Boorstein and Ewald, 1987) Nevertheless, the adaptive significance of fever in ectotherms is still debated Various pyrogens failed to induce fevers in several species of reptile (Laburn *et al*, 1981; Zurovsky *et al*, 1987a, b) Blatteis (1986) argues that generalizations about the evolutionary significance and beneficial consequences of fevers are premature without further study.

If fever is adaptive for ectotherms, related physiological adjustments might be expected to accompany behavioural hyperthermia In this study, we examined whether febrile toads, *Bufo marinus*, exhibited concomitant changes in thermal tolerance Increases in thermal tolerance might be advantageous to febrile ectotherms which expose themselves to warmer microclimates We chose the tropical to subtropical toad *B marinus* because it can exploit hot, dry environments and possesses physiological capacities to cope with such stresses (Sherman, 1980)

MATERIALS AND METHODS

Male and female *B marinus* (100–300 g) were obtained from a commercial supplier Prior to experiments, toads were maintained unfed for at least 1 week at 25.0 (±1 0)°C on a 12 12 L D photoperiod (centred at noon EST) in aquaria with access to both dry areas and free water

Pyrogen

Several species of amphibian develop behavioural fevers in response to injection with the gram-negative bacterium *Aeromonas hydrophila* (Casterlin and Reynolds, 1977, Kluger, 1977) The pyrogenicity of all gram-negative bacteria derives from the lipopolysaccharide (LPS) in their cell walls (Zurovsky *et al*, 1987a). We thought it likely, therefore, that toads would develop behavioural fevers in response to LPS injections The LPS (supplied by Sigma Chemical Co, extracted from *Escherichia coli* Serotype 0127 B8) was prepared under sterile conditions at a concentration of 15 mg/ml of 0 9% saline made using sterile, pyrogen-free water The volume injected was adjusted for mass so that each toad received 1 mg LPS/50 g wet mass (see Bronstein and Conner, 1984) Comparable volumes of saline were injected into control toads

Behavioural fever

Saline-injected and LPS-injected toads were placed individually on a thermal gradient before and after injection and selected temperatures were observed to allow comparison of changes in preference Each toad was injected subcutaneously and placed in the gradient at 1000 EST to remove effects of possible circadian variation on selected temperature

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The thermal gradient was established in an insulated wooden box (175 × 18 × 15 cm) the floor of which was a steel plate extending 12 cm from both ends of the box. The gradient was achieved by placing one end of the steel plate on a hot plate and the other end in a styrofoam box filled with dry ice During an experiment, the steel plate was covered with 2–3 cm of moist vermiculite in which YSI thermistors were embedded every 4 cm The YSI thermistors were connected to a YSI Model 47 Telethermometer The extent of the gradient ranged from −2 to 50°C Body temperature was monitored with another YSI thermistor inserted into the lower intestine of the toad and fixed in place with a string harness This thermistor was connected to an Apple IIe computer which monitored body temperature every 5 min The thermistor did not appear to impede the toad's movement within the gradient.

There is considerable intraspecific variation in selected body temperatures of amphibians (Brattstrom, 1970). Therefore, each toad was used as its own control One day prior to injection, each toad was placed in the middle of the gradient and its selected temperature was monitored for 5 h, after which it was returned to its maintenance tank Following injection with either LPS or saline, each toad was placed in the middle of the gradient and its selected temperature was monitored for 11 h

Mean selected temperatures before and after injection were calculated for each animal In our calculation of mean selected temperature, we did not want to include potentially misleading temperatures derived from "exploratory" behaviour at the beginning of each experiment To insure that the observations used reflected thermoregulatory behaviour, we calculated standard deviations for overlapping, successive 30 min segments of the experiment (centred on each temperature reading) and used data in the calculation of mean selected temperature beginning with the first time that the standard deviation fell to 0 5 or below Behavioural fever would be manifested as an increase in mean selected temperature in pyrogen-injected toads compared to the change in mean selected temperature in the saline-injected toads Seven pyrogen-injected toads and five saline-injected toads were used in this part of the study Differences in mean selected temperatures before and after injection were determined using the Student's *t*-test

Thermal tolerance

The index of thermal tolerance we used was the critical thermal maximum (CTM), defined as the temperature beyond which animals become so uncoordinated as to lose all capacity to escape from conditions that will soon lead to their death (Lowe and Vance, 1955) Loss of righting ability was a reliable indicator of CTM

Four treatment groups were established comprising toads injected with either saline or pyrogen and then maintained at either 25 0 (±1 0)°C or 32 0 (±1 0)°C for 8 h prior to the CTM determination We chose 25°C as the normothermic incubating temperature because Brattstrom (1963) reported that the mean body temperature of free-ranging *B marinus* was 25 2°C We chose 32°C as the febrile incubating temperature because it was close to the mean maximum selected temperature of pyrogen-injected toads in this study Eight hours was well within the period during which the LPS-injected toads expressed behavioural fever

During CTM determination, each toad was placed individually in a vessel containing 6 litres of water The water was heated on a hot plate at a rate of 0 5–1 0°C/min, and a power stirrer kept the water well-mixed. Body temperature was measured as described above with a YSI thermistor fixed in the lower intestine of the toad and connected to an Apple IIe computer. The temperature at which loss of righting ability occurred was recorded There were between 6 and 9 toads in each of the four treatment groups and all individuals recovered from this procedure

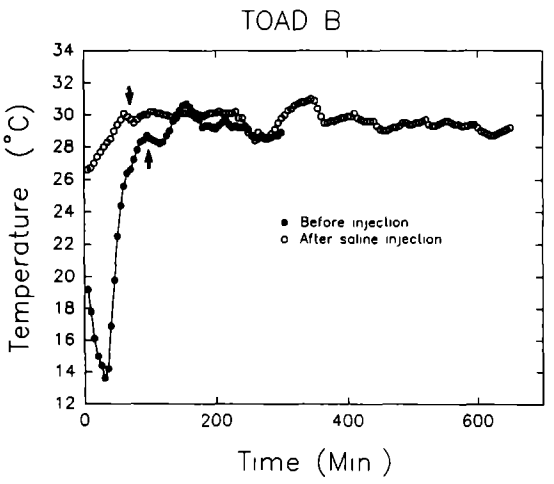

Fig 1 Selected temperature over time of a toad on the thermal gradient before (●) and after (○) injection with saline Arrows indicate points beyond which data were used in the calculation of mean selected temperature (see Methods section)

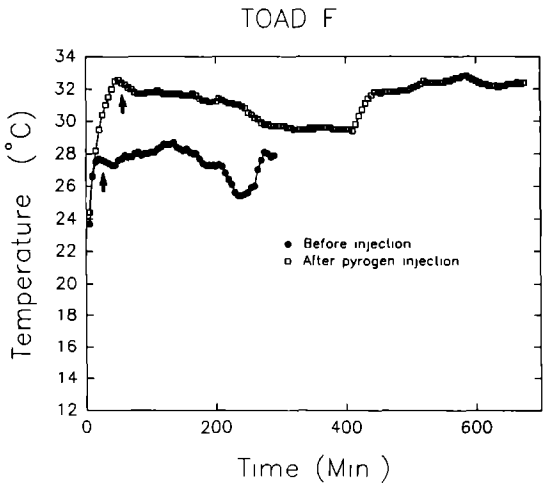

Fig 2 Selected temperature over time of a toad on the thermal gradient before (●) and after (□) injection with pyrogen Arrows indicate points beyond which data were used in the calculation of mean selected temperature (see Methods section)

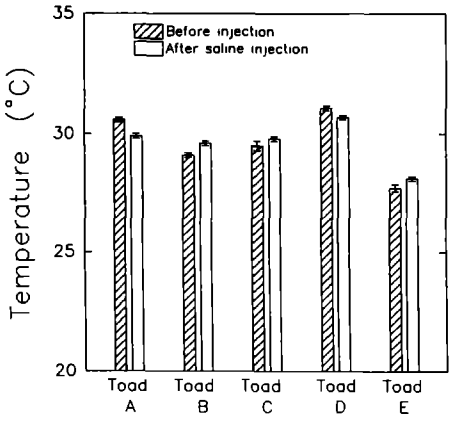

Fig 3 Mean selected temperature (±SEM) for each of 5 toads before (shaded bars) and after (unshaded bars) injection with saline

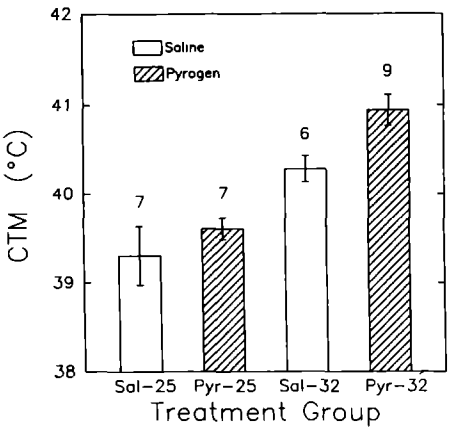

Fig 5 Mean CTM (±SEM) of toads injected with either saline (unshaded bars) or pyrogen (shaded bars) and then incubated for 8 h at either 25 or 32°C Numbers indicate sample sizes of the treatment groups

Differences in mean CTMs were determined using Student's *t*-test

RESULTS

Figure 1 shows selected body temperature of a typical toad on the thermal gradient before and after injection with saline. Prior to injection, this toad exhibited a good deal of "exploratory" behaviour before settling down to its mean selected temperature of 29 1 ± 0.1°C (mean ± SEM) Following saline injection on day 2, the toad exhibited considerably less exploratory behaviour and its mean selected temperature was little changed from the previous day (29.6 ± 0.1°C) Figure 2 shows the selected body temperature of a toad on the thermal gradient before and after injection with LPS. The mean selected temperature of this toad was 27 4 (±0.1)°C before injection and 31.2 (±0.1)°C after injection.

Saline injection produced no consistent change in mean selected body temperature ($P > 0.5$, Fig 3). The average change in mean selected temperature after injection with saline was +0.02°C The mean minimum and maximum selected temperatures of the toads prior to injection with saline were 28.3 (±0.7)°C and 30.8 (±0.7)°C, respectively The mean minimum and maximum selected temperatures of the toads following saline injection were 27 4 (±0 5)°C and 31.2 (±0 5)°C, respectively

Toads injected with pyrogen exhibited a statistically significant increase of 2 2°C in mean selected temperature ($P < 0.01$, Fig 4). The mean selected temperature of 27 5 (±0.6)°C prior to injection increased to 29.7 (±0 5)°C following pyrogen injection The mean minimum and maximum selected temperatures of the toads prior to injection with pyrogen were 25 1 (±0.9)°C and 29 8 (±0 5)°C, respectively Following LPS injection, the mean minimum and maximum temperatures of the toads were 27 5 (±0 8)°C and 31 1(±0.4)°C, respectively The onset of fever occurred between 1 and 3½ h after LPS injection (average latency was about 2¼ h) and persisted through the end of the 11 h experiment

Toad I did not develop a fever following injection with LPS (Fig. 4) In fact, its mean selected temperature decreased from 29.3°C prior to injection to 27 9°C following injection.

The difference in CTM between saline-injected toads (39.3 ± 0 3°C) and pyrogen-injected toads (39.6 ± 0.1°C) incubated at 25°C was not significant ($P > 0$ 2, Fig. 5). However, the CTM increased significantly from 40 3 (±0 2)°C for saline-injected toads to 40 9 (±0 2)°C for pyrogen-injected toads

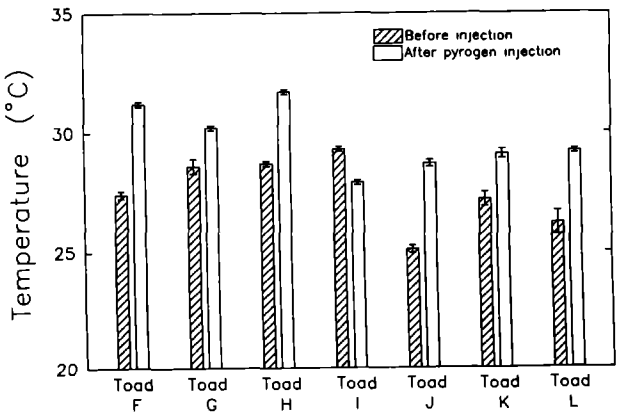

Fig 4 Mean selected temperature (±SEM) for each of 7 toads before (shaded bars) and after (unshaded bars) injection with pyrogen

if the animals were incubated at 32°C ($P < 0.025$, Fig 5)

DISCUSSION

This study is the first to demonstrate LPS-induced behavioural fever in an amphibian Behavioural fever was expressed as a 2.2°C increase in mean selected temperature with a latency of about $2\frac{1}{4}$ h following injection with LPS The fever induced by LPS In *B marinus* is comparable to that induced by the bacterium *Aeromonas hydrophila* in other amphibians Casterlin and Reynolds (1977) reported an increase in preferred temperature of 2.6 and 2.7°C in febrile larval *Rana catesbeiana* and *R pipiens*, respectively Infected *Hyla cinerea* exhibited a fever of over 2°C with a latency of about 2–4 h (Kluger, 1977) The similarity between fevers induced by LPS and *A hydrophila* is consistent with the fact that LPS is the pyrogenic agent of gram-negative bacteria (Zurovsky *et al*, 1987a)

Kluger (1977) noted that, unlike the febrile response of reptiles, there was considerable variation in the thermoregulatory response of infected *H cinerea* Only 15 of the 31 infected frogs in that study exhibited an increase in mean selected temperature of 1°C or more Twelve frogs exhibited no change in selected temperature and 4 frogs became hypothermic by 1°C or more The response of toads to LPS injection in our study varied as well Six toads developed a behavioural fever and raised their body temperature an average of 2.8°C while the mean selected temperature of one toad decreased by 1.4°C (Fig 4) Blatteis (1986) noted that a number of mammals become hypothermic in response to LPS injection The causes of intraspecific and interspecific variation in the febrile response are unknown (Blatteis, 1986)

The CTM of 39.3°C of saline-injected *B marinus* held at 25°C was identical to the CTM reported by Brattstrom (1968) for *B marinus* acclimated to 26°C The CTM of pyrogen-treated toads increased significantly over that of control toads if both groups of toads were incubated at a febrile temperature but not if they were incubated at a normothermic temperature (Fig 5). An increase in thermal tolerance would be advantageous to infected animals which tend to seek out warmer microclimates The toads in this study never exposed themselves on the thermal gradient to temperatures warmer than about 8°C below CTM However, the temperatures of microclimates that animals might exploit in the wild in order to achieve behavioural fever might be less predictable than on a thermal gradient An elevated CTM would be advantageous to an infected animal exploiting a warmer microclimate which could become dangerously hot Whether or not levels of thermal incapacitation less severe than loss of righting occur at higher temperatures in febrile, infected animals deserves investigation

Kluger (1986) noted that a number of immune functions of infected animals are enhanced by a transient, modest increase in body temperature Similarly, the elevated CTM of LPS-treated toads reported in our study occurred only if the toads were hyperthermic Other related physiological adjustments may occur in pyrogen-treated ectotherms if they are allowed to become hyperthermic Malvin and Kluger (1979) reported that the oxygen consumption rate of infected iguanas maintained at afebrile temperatures was not significantly different from that of control animals They concluded that in spite of the elevated thermal set point that occurs during fever, iguanas do not increase their metabolic heat production The iguanas, however, were not permitted to raise their body temperature There are significant metabolic costs associated with fever in mammals (Kluger, 1986) Increases in metabolic rate that might be associated with the febrile response of ectotherms may not occur unless animals are permitted to maintain elevated body temperatures

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