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BEHAVIOURAL FEVER IN A NAMIB DESERT TENEBRIONID BEETLE, *ONYMACRIS PLANA*

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Abstract—The Namib Desert beetle, *Onymacris plana* developed a behavioural fever when given injection of highly purified lipopolysaccharide isolated from *E. coli*. The beetles sought a higher preferred temperature in a thermal gradient after injection of the pyrogen than they had previously chosen. The elevation of thermal preference was observed within 1 h after injection and remained elevated for at least 12 h. The peak amplitude was 1.5°C, about 5 h after injection of lipopolysaccharide doses of around 250 mg . kg^{-1}. This is the second report of febrogenesis in an insect and the first using a highly purified lipopolysaccharide pyrogen.

Key Word Index: Behavioural fever, thermal biology, arthropod fever, thermal preference, purified lipopolysaccharide

INTRODUCTION

To date a number of Arthropod species have been shown to develop a behavioural fever in response to injection of bacterial endotoxins or prostaglandins of the *E* series (Table 1). However, only recently has febrogenesis in an insect been reported. The Madagascar cockroach, *Gromphadorhina portentosa* when injected with either endotoxin from heat killed *E. coli* or lipopolysaccharide *W* will seek a higher temperature in a thermal gradient which is most pronounced 19 h after injection (Bronstein and Conner, 1984). This increase in the thermal preferendum is known as a behavioural fever and is characteristic of the ectotherms so far examined for the febrile response.

Behavioural fever in Arthropods can be detected usually in less than 1 h with a peak amplitude anywhere from 2 to 19 h after injection (Table 1). The degree of change varies with the dosage of pyrogen and the species in question. For example, in the crayfish, *Cambarus bartoni*, the thermal preference shifted only 1°C at a low dosage of prostaglandin E_1 (Casterlin and Reynolds, 1978). However, the scorpion, *Buthus occitanus* shifted its thermal preference up to 20°C above normal with injection of prostaglandin E_1 (Cabanac and Le Guelte, 1980). The duration of the behavioural fever in Arthropods is often 24 h and in the case of the cockroach, *Gromphadorhina portentosa* it remained above its normal thermal preference for up to 30 h.

It appears that behavioural fever in arthropods is widespread and might even be ubiquitous amongst the invertebrates. But until studies are done on a number of different insect species representing the various orders as well as on other arthropods, the phylogeny of the febrile response and its purported survival value (Kluger, 1979) will remain in question.

Lipopolysaccharides from Gram-negative bacteria are potent pyrogens in mammals. Their degree of pyrogenicity is somewhat obscured by preparations not being highly purified. The commercially supplied lipopolysaccharide (Sigma and Difco) contain significant amounts of protein, RNA, and dialysable contaminants (Warner *et al.*, 1985). The dosages of pyrogens used thus far for Arthropod species in order to be effective have far exceeded those necessary to be fever causing in mammals (mg . kg^{-1} rather than ng . kg^{-1}). We therefore used highly purified lipopolysaccharide to ascertain whether the magnitude of difference might not be due to the impurities in the pyrogen having unknown effects.

We report here on the behavioural fever developed by the Namib Desert beetle, *Onymacris plana* after injections of highly purified lipopolysaccharide isolated from *E. coli*. *O. plana* is a large apterous diurnal beetle ideally suited for investigation of febrogenesis. It is substrate dependent and chooses a narrow temperature preference when placed in a thermal gradient. Shifts in the thermal preferendum are therefore easily detected.

MATERIALS AND METHODS

Lipopolysaccharide purification and injection

Phenol-extracted lipopolysaccharide from *E. coli* 0111:B4 was obtained from Sigma Chemical Company (St Louis, MO). It was purified according to the method of Warner *et al.*, 1985. The purified lipopolysaccharide was dissolved in sterile pyrogen-free 0.7% saline which was isomotic with the haemolymph.

Control beetles were injected with 10 μl of 0.7% pyrogen-free saline. Experimental beetles were injected with lipopolysaccharide solutions of various concentrations ranging from 1 to 20 mg . ml^{-1} made up

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Table 1. Behavioural fevers in arthropods

Arthropod Species	Pyrogen	ΔT(°C)	Onset	Peak	Duration	Authors
Scorpion						
Buthus occitanus	PGE_1	5–20°C	< 1 h	2 h	20 h	Cabanac and Le Guelte (1980)
Androctonus autralis	PGE_1	5–15°C	< 1 h	2 h	20 h	Cabanac and Le Guelte (1980)
Crayfish						
Cambarus bartoni	Killed bacteria	1.8°C	—	—	24 h	Casterlin and Reynolds (1977) (1980)
	PGE_1	1–3.4°C	—	—	24 h	Casterlin and Reynolds (1978)
Lobster						
Homarus americanus	PGE_1	4.7°C	< 1 h	4–8 h	24 h	Casterlin and Reynolds (1979)
Horseshoe crab						
Limulus polyphenus	PGE_1	6.0°C	< 1 h	4–8 h	24 h	Casterlin and Reynolds (1979)
Shrimp						
Penaeus duorarum	PGE_1	4.5°C	< 1 h	4–8 h	24 h	Casterlin and Reynolds (1979)
Madagascar cockroach						
Gromphadorhina portentosa	Killed bacteria	3.6°C	< 4 h	19 h	30 h	Bronstein and Conner (1984)
	Endotoxin	4.2°C	16 h	29 h	24 h	Bronstein and Conner (1984)
Tenebrionid Beetle						
Onymacris plana	Purified endotoxin	1.5°C	< 1 h	5 h	12 h	Present study

in 10 μl of 0.7% pyrogen-free saline. When calculated in terms of the hydrated mass of each beetle, these lipopolysaccharide dosages ranged from 1–400 mg . kg^{-1} along a continuum. To facilitate analysis this dose range was divided into four dose categories with approximately equal number of beetles in each, i.e. 1–99 mg . kg^{-1}; 100–199 mg . kg^{-1}; 200–299 mg . kg^{-1} and 300–399 mg . kg^{-1}. A 50 μl Hamilton syringe was used for all injections. The site of the injection was near the anus. *O. plana* has an unusually thick cuticle and in order to prevent leakage of the solutions the needle was inserted carefully alongside the anus and pushed gently into the haemocael. Care was taken not to damage the internal organs. A group of *O. plana* were injected employing the above procedure with solutions containing Evans blue dye. The dye was progressively cleared from the haemolymph and concentrated in the Malpighian tubules. This corroborated the fact that the lipopolysaccharide solutions were freely circulating in the haemocoel after injection.

Animals

Onymacris plana was collected in the Namib Desert in South West Africa/Namibia. They were housed in our animal care facilities at the University of the Witwatersrand Medical School on a 12 h light–12 h dark cycle. They lived on their sand substrate and were fed a diet of lettuce and oats. Adults of both sexes with similar numbers in each group were used. The adult of *O. plana* is from 1 to 2 cm long and weighs from 0.50 to 1.50 g.

Thermal gradient

Thermal preferences for each beetle were established by means of a thermal gradient chamber. This consisted of a 2 m-long wooden box, the floor of which was made of hardboard laid down over desert sand. A sloping bank of incandescent lamps was suspended over the chamber along its length. These lamps and a compartment containing ice at one end of the chamber provided a temperature gradient along the length of the chamber. A fluorescent light suspended alongside the chamber ensured even lighting throughout. The thermal gradient along the length of the chamber was measured during each experiment by means of a calibrated Bailey Thermalert TH-6 thermometer (Bailey Instruments, Saddle Brook, NY). Surface temperatures along the length of the chamber ranged from 20 to 90°C, giving rise to a smoothly progressing and consistent gradient. Sand surface temperatures in the Namib Desert often reach 70°C during the summer months. The gradient temperatures encompassed such extremes. This gradient provided a convenient, non-invasive method of assessing the surface temperture encountered by a beetle at any time. To ascertain the body temperature, thirty-guage (copper-constantan) indwelling thermocouples were implanted through the pronotum into the thoracic musculature of 10 beetles while in the gradient. The thoracic temperatures were found to be 4°C higher than thermal gradient surface temperatures no matter the beetles' position in the gradient. These measurements enabled us to estimate the body temperature for any given surface temperature that a beetle would choose. At all times, the beetles had access to fresh lettuce distributed evenly throughout the chamber to ensure adequate hydration. No attempt was made to control for humidity as these diurnal beetles are normally subjected to variable humidities under desert conditions (Louw and Seely, 1982). A 24 h period of acclimation for the beetles to the thermal gradient in the chamber preceded any measurements for the beetles thermal preference. Up to thirty beetles at a time could be conveniently observed. There was little social behaviour and minimal interference noted. Each beetle was marked by means of a number painted on the surface of the dorsal elytra with water-soluble paint. All beetles were introduced into the chamber at 10:00 a.m. and care was taken to account for circadian rhythms by adhering to a fixed time schedule for all experiments. The following day, at 10-min intervals from 13:00 h until 18:00 h measurements

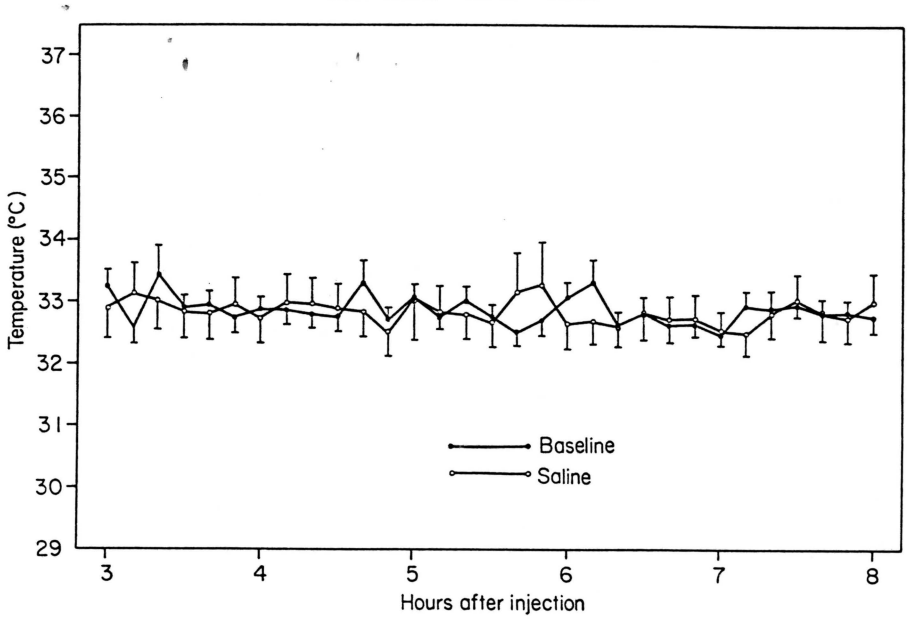

Fig. 1. Means and Standard Errors for the thermal preference data obtained from beetles injected with a 0.7% Pyrogen-free Saline solution, superimposed on the data obtained from beetles under baseline conditions. ($n = 25$ for Saline, and $n = 162$ for baseline).

were done of baseline thermal preferences. If high doses of lipopolysaccharide were to be injected into the beetles, measurements were made every 10 min from 10:00 a.m. until 18:00 h and every half hour past 18:00 h until 10:00 a.m. the next day. Once the baseline thermal preference had been established for each beetle, it received a 10 μl injection of either 0.7% pyrogen-free saline, or a lipopolysaccharide solution, prior to redetermination of its thermal preference. Since surface temperatures along the gradient were known each beetle's mid-point could be noted at 10 min intervals and the concomitant temperature recorded.

162 beetles were used for these experiments. Statistical analysis was performed using the Anova- and -one way procedures on SPSS-X. In all tests of significant differences a $P < 0.001$ was taken as the critical value.

RESULTS

O. plana adults of both sexes sought that portion of the gradient where the surface temperature was between 33 and 34°C (Fig. 1). The corresponding thoracic temperatures were therefore between 37 and 38°C. Field studies in the desert corroborate these findings. Under natural conditions *O. plana* has been shown behaviourally to maintain its body temperature between 36 and 38°C for most of the day (McClain, unpublished work). A behavioural fever would be reflected in a choice of temperatures above the normal field or laboratory determined ones.

Injections of 10 μl of 0.7% pyrogen-free saline did not alter the thermal preference (Fig. 1). No significant differences could be found (even for $P < 0.05$) between the thermal preferenda of baseline and saline injected beetles or between thermal preferenda of baseline and lipopolysaccharide doses less than 100 mg . kg^{-1}. At a higher dosage of lipopolysaccharide (100–199 mg . kg^{-1}) the beetles sought a higher temperature within the first hour. This was dramatic for the first 3 h after which time the insects sought and maintained a behavioural fever of at least 1°C above normal for the next 8 h (Fig. 2). In Figure 3 results from the 200–299 mg . kg^{-1} dosages are shown. Again the thermal preferenda shifted within the first hour and remained up to 1.5°C above normal until 12 h after injection. At the highest dosage of lipopolysaccharide injected (300–399 mg . kg^{-1}) the elevation of thermal preference was not as marked as for doses between 100 and 300 mg . kg^{-1} (Fig. 4).

A two-way Analysis of Variance across time and the various dosage groups revealed that significant differences existed between the thermal preferenda of beetles when baseline values were compared with experimental conditions. The Scheffe test was used to clarify between which groups the significances existed. In this test time was not considered.

DISCUSSION

The results from these experiments indicate that *O. plana* exhibits a behavioural fever when injected with highly purified lipopolysaccharide from *E. coli*. The pyrogen has its greatest effect in the dose range of 100 mg . kg^{-1} to 300 mg . kg^{-1}. The beetles sought a higher temperature within the first hour with a peak appearing 5 h after injection. An increase of about 1.5°C in preferred temperature lasting up to 12 h was observed. The scorpions, lobster, horseshoe crab, shrimp and cockroach all showed a similar rapid onset (Table 1). The peak of the behavioural fever (5 h) was similar to the lobster, horseshoe crab and shrimp (4–8 h) rather than to the cockroach (19 h). Of all the Arthropod species so far examined, *O. plana* exhibits a behavioural fever of the shortest duration. Thermal preference was significantly raised for only

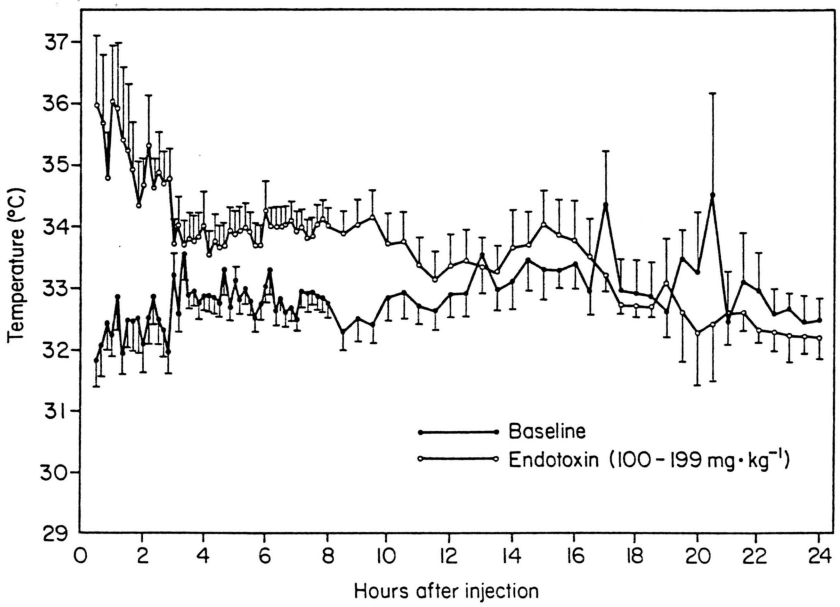

Fig. 2. Means and Standard Errors for the thermal preference data obtained from beetles injected with lipopolysaccharide solution of 100–199 mg . kg^{-1}, superimposed on the data obtained from beetles under baseline conditions. For lipopolysaccharide, $n = 14$, 35 and 14 for 0–3, 3–8 and 8–24 h after injection respectively. ($n = 162$ for baseline).

about 12 h before returning to baseline values. Other workers report between 20 to 24 h or even longer for elevated thermal preferences. The cockroach remained well above its thermal preference for up to 30 h after injection (Bronstein and Conner, 1984). No comparison can be made with lipopolysaccharide injection between these two species of insect as the onset of the behavioural fever in the cockroach after injection of lipopolysaccharide was after 13 h. Our data show that *O. plana* will seek a higher temperature after only 1 h after injection of the pyrogen.

Recent work by Marx *et al.*, 1985 and Laburn *et al.*, 1981 have shown that endotoxin and other pyrogens have failed to induce behavioural fever in ectotherms such as fish and lizards. The implication being that the purported "behavioural fevers" which are observed in these organisms are nothing more than immobilizing effects on the thermoregulatory centres,

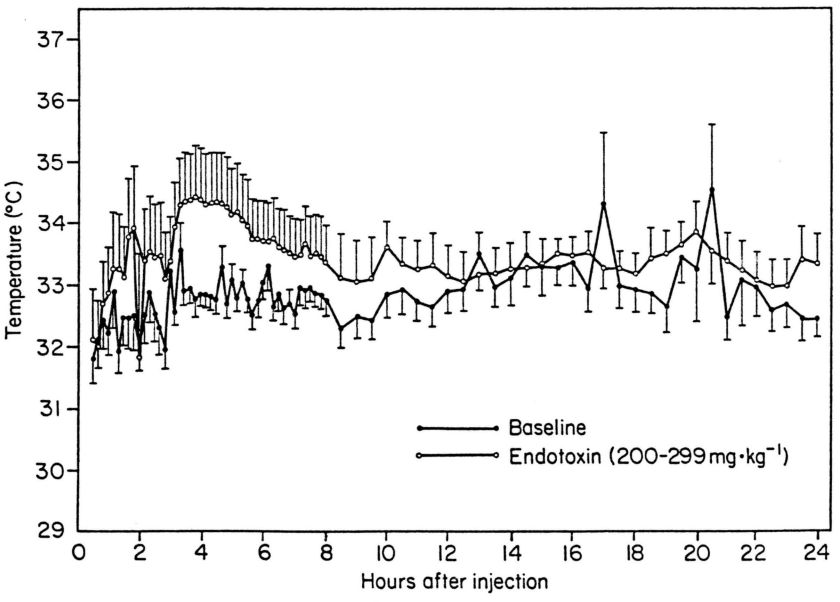

Fig. 3. Means and Standard Errors for the thermal preference data obtained from beetles injected with lipopolysaccharide solutions of 200–299 mg . kg^{-1}, superimposed on the data obtained from beetles under baseline conditions. For lipopolysaccharide, $n = 11$, 13, and 11 for 0–3, 3–8, 8–24 h after injection respectively. ($n = 162$ for baseline).

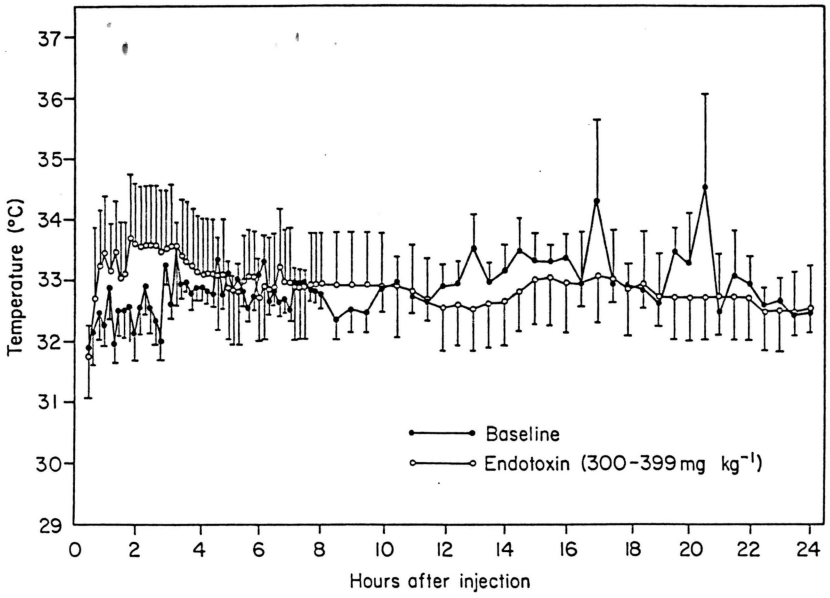

Fig. 4. Mean Standard Errors for the thermal preference data obtained from beetles injected with lipopolysaccharide solutions of 300–399 mg . kg^{-1}, superimposed on the data obtained from beetles under baseline conditions. For lipopolysaccharide $n = 13$, 13 and 11 for 0–3, 3–8. 9–24 h after injection respectively. ($n = 162$ for baseline).

brought about by the ectotherms being paralyzed by the pyrogen (Marx *et al.*, 1985). At higher doses of lipopolysaccharide some of the beetles appeared lethargic for a few hours after injection. This could account for the seemingly anomolous elevation of thermal preference in the 100–199 mg . kg^{-1} dosage during the first three hours after injection (Fig. 2). However, the beetles appeared once again to be able to thermoregulate as they maintained their body temperature at the new thermal preference then re-established their normally preferred temperature. Much more work on behavioural fever needs to be done in order to clarify this phenomenon.

Increasing doses of our highly purified lipopolysaccharide, from *E. coli*, injected into *O. plana* affected the magnitude of the thermal response (Figs 2 and 3), except at the highest dose. The first 2 h elevated thermal preference of the 100–199 mg . kg^{-1} injection of the lipopolysaccharide (Fig. 2) could be due to the immobilizating effects of the pyrogen and were therefore not considered in the dose–response. Similarly *Gromphadorhina portentosa* injected with increasing doses of heat-killed *E. coli* shifted its thermal preference maximally with the highest dose (Bronstein and Conner, 1984). A comparable effect was noted with injections of high dosages of prostaglandin E_1 in scorpions (Cabanac and Le Guelte, 1980). It would appear that the overt response to seek a high temperature by the arthropod is in accordance with some survival value, as has been documented elsewhere (Kluger, 1979).

The purification of commercially available lipopolysaccharide did not reduce the extremely high concentrations of the pyrogen necessary to bring about and sustain the behavioural fever in *O. plana*. The lipopolysaccharide is derived from the cell walls of Gram-negative bacteria and the pyrogenicity of lipopolysaccharide is mediated by the lipid *A* moiety of the molecule (Luderitz *et al.*, 1966). Both in the cockroach and now in *O. plana* a behavioural fever results from injection of this pyrogen. In the cockroach injection of heat-killed bacteria (*E. coli*) and the lipopolysaccharide derived from *E. coli* produced the peak response at roughly similar times (Bronstein and Conner, 1984), indicating that pyrogenicity resides in the lipopolysaccharide molecule. The results from the present study with *O. plana* corroborate the lipopolysaccharide findings in the cockroach and support the notion of a febrile response in Arthropods. However, combined studies on the insect immune system with concomitant febrile response to pyrogens needs to be done to substantiate fever as ubiquitous in invertebrates or in ectotherms.

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Note in proof:

Boonstein S. M. and Ewald P. W. (1987) Costs and benefits of behavioural fever in *Melanoplus sanguinipes* infected by *Nosema acridophagus*. *Physiol. Zool.* **60**(5), 586–595.

The inoculated the grasshopper *Melanoplus sanguinipes* with the protozoan *Nosema acridophagus* which resulted in an increase in the grasshopper's preferred temperature by about 6°C.