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PYROGENS FAIL TO PRODUCE FEVER IN THE SNAKES *PSAMMOPHIS PHILLIPSII* AND *LAMPROPHIS FULIGINOSUS*

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Abstract—1. Preferred body temperature of five diurnal, *Psammophis phillipsii* and three nocturnal, *Lamprophis fuliginosus*, snakes was measured in a thermal gradient chamber by indwelling colonic thermocouples, before and after injection of a variety of pyrogens.

2. The snakes achieved their preferred body temperature by moving up and down in the gradient chamber; it was about 33°C for *P. phillipsii* and 25°C for *L. fuliginosus*.

3. The snakes did not develop fever in response to any of the pyrogens, whether gram-negative or gram-positive in origin, either on the day of injection or on the subsequent day.

4. We believe that fever is rare amongst reptiles.

INTRODUCTION

Fever has been observed in species from several different orders of ectotherm (Kluger, 1979), but the question of how ubiquitous fever is amongst ectotherms has not been resolved. Of all the ectotherms, the reptiles are the most likely to develop fever: they have a larger thermal inertia than other terrestrial ectotherms (which reduces short-term variations in body temperature), they have the capacity to regulate body temperature behaviourally, and at least some species have autonomic thermoregulatory mechanisms too (Spellerberg, 1982). Also, reptiles have access to heterogeneous thermal environments, an advantage for behavioural thermoregulation. An assessment of how ubiquitous fever is, therefore, could begin with profit with the reptile.

Most investigations of the effects of pyrogens on reptiles have employed lizards. Following injection of a gram-negative pyrogen, behavioural fever develops in three species of lizards *Dipsosaurus dorsalis* (Bernheim and Kluger, 1976), *Iguana iguana* (Grieger and Kluger, 1978) and *Crotaphytus collaris* (Firth *et al.*, 1980). All three species belong to the family Iguanidae. We have shown that gram-negative pyrogens do not induce fever in the cordylid lizard *Cordylus cataphractus*, an excellent behavioural thermoregulator (Laburn *et al.*, 1981), and we have evidence (unpublished observations) that members of four other families of Old World lizards also do not respond to gram-negative pyrogen. In addition, we have studied one reptile which is not a lizard, the tortoise *Geochelone pardalis*, and it does not develop fever in response to either gram-negative or gram-positive pyrogens (Zurovsky *et al.*, 1987).

We report here an extension of the search for fever amongst reptiles to two species of snake, the olive grass snake (*Psammophis phillipsii*), which is known for its sunbasking behaviour, and the crepuscular to nocturnal brown house snake *Lamprophis fuliginosus*.

MATERIALS AND METHODS

Animals

The snakes (*P. phillipsii*, $n = 5$; *L. fuliginosus*, $n = 3$) were obtained from the Transvaal Division of Nature Conservation, and were held in sand-based enclosures at a controlled air temperature of 27 ± 0.5°C for at least 2 weeks prior to experimentation. Each enclosure contained a tungsten lamp, providing the opportunity for behavioural thermoregulation. The snakes weighed 250 g–410 g (*P. phillipsii*) and 64 g–130 g (*L. fuliginosus*). They were fed live baby mice once a week, and had access to water *ad libitum*.

Photothermal gradient chamber

During experiments the snakes were exposed individually in a photothermal gradient chamber with a sand substrate. The walls of the chamber were made of asbestos cement, and the top was sealed with perspex (*P. phillipsii* is venomous); chamber dimensions were about 1.8 m long, 0.2 m wide and 0.3 m high.

The chamber was placed in an air-conditioned room. Heat was provided to one end by means of tungsten lamps. During experiments with *P. phillipsii*, the temperature at the hot end of the chamber, measured with a thermocouple in a blackened copper tube, was 58–60°C; during experiments with *L. fuliginosus* it was 69–73°C. The temperature at the cold end, measured by means of an exposed thermocouple, was 24–26°C and 6–10°C respectively. Greater extremes of temperature were provided for the crepuscular/nocturnal *L. fuliginosus*, by packing dry ice around the cold end of the chamber.

Body temperature

The body temperature of the snakes was measured by means of an indwelling copper–constantan thermocouple inserted into the colon via the cloaca to a depth of 30–50 mm. The thermocouple was taped to the snake's tail just caudal to the cloaca. Restriction of general mobility was minimal. The thermocouple output was detected on a data logger (Esterline Angus PD2064).

The responses of *P. phillipsii* to injections were expressed as the mean changes in colonic temperature and the 9-hr thermal response index (TRI9), which is the time integral, over 9 hr, of the rise of colonic temperature following

injection (Clark and Cumby, 1975). The responses of *L. fuliginosus* were expressed as mean colonic temperature.

Injections

All injections were made into the peritoneum, through the ventral body wall, slightly caudal to the mid-point of the snake's length.

Pyrogens

The following pyrogens were used for *P. phillipsii*: lipopolysaccharide (LPS) extracted from *Salmonella typhosa* (Difco) at concentrations of 1 and 10 μg/kg; killed organisms of *Staphylococcus aureus* (Pansorbin, Calbiochem) at a concentration of 5×10^7 organisms/kg; killed organisms of *Salmonella minnesota* at a concentration of 5×10^7 organisms/kg. In *L. fuliginosus* we injected only LPS (10 μg/kg). Control injections in *P. phillipsii* consisted of 1 ml of sterile saline, and in *L. fuliginosus* 1 ml of sterile water. All pyrogens were checked for activity by intravenous injection in conscious rabbits.

Experimental procedure

Snakes were investigated one at a time. *P. phillipsii* was kept in the gradient chamber for 24 hr before investigation, and *L. fuliginosus* for 2 hr. All injections in the diurnal *P. phillipsii* were made at 07.30, and in the nocturnal *L. fuliginosus* at 18.00. The heat lamps were switched on at 07.30, and left on throughout all exposures.

After injections were made, the snakes were placed in the middle of the gradient. Body temperatures were monitored at 10 min intervals. Temperature measurements also were made on the day following that of injection.

Statistical analyses

Data were subjected to Student's t test, with the Bonferroni correction for repeated measures where applicable. Level of significance was taken to be 5%.

RESULTS

The snakes were active and mobile following all injections. They moved freely up and down the gradient.

Figure 1 shows the colonic temperature of one *P. phillipsii* following saline injection, and the pattern is characteristic of all that species' responses. At the beginning of the exposure the snake's temperature was equal to air temperature. Temperature then rose rapidly as the snake exploited the radiant heat of the lamps, and, after some oscillation, settled at a plateau which persisted for several hours. The mean plateau temperature following saline injection was 33.5 ± 2.1°C (mean $\pm$ SD, $n = 5$, range 28.0–40.3°C). By contrast, the mean colonic temperature of *L. fuliginosus* following saline injection, with the lamp on, was 25.4 ± 1.0°C (mean $\pm$ SD, $n = 3$, range 24.0–27.0°C).

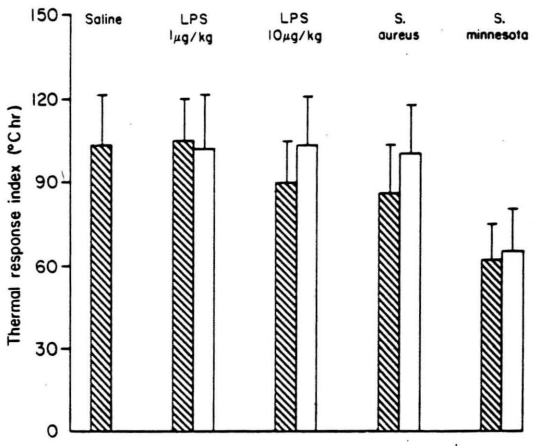

Fig. 2. Nine hour thermal response index (mean $\pm$ SD) for five snakes (*P. phillipsii*) given sterile saline, 1 μg/kg lipopolysaccharide, 10 μg/kg lipopolysaccharide, 5×10^7 organisms/kg of killed *S. aureus* and 5×10^7 organisms/kg of killed *S. minnesota*. Hatched columns are values for the day of injection, open columns for the day following injection.

Figure 2 shows the thermal response index for *P. phillipsii* following saline injection and the injection of the various pyrogens, on the day of injection and the subsequent day. The TRI9 following pyrogen injection was not significantly greater for any pyrogen, than the TRI9 following saline injection. On both days following *S. minnesota* injection, it was significantly lower.

Figure 3 shows the maximum colonic temperature of *P. phillipsii* following each of the injections. Again

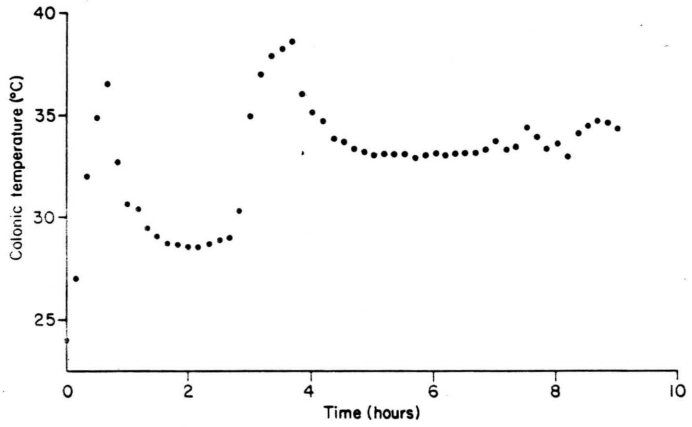

Fig. 1. Colonic temperature of one snake (*P. phillipsii*) nine hours following intraperitoneal injection of 1 ml sterile saline at $t = 0$.

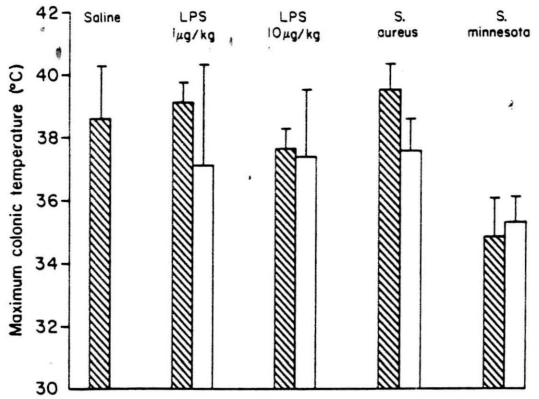

Fig. 3. Maximum colonic temperature (mean ± SD) for five snakes (*P. phillipsii*) given sterile saline, 1 μg/kg lipopolysaccharide, 10 μg/kg lipopolysaccharide, 5×10^7 organisms/kg of killed *S. aureus* and 5×10^7 organisms/kg of killed *S. minnesota*. Hatched columns of values for the day of injection, open columns for the day following injection.

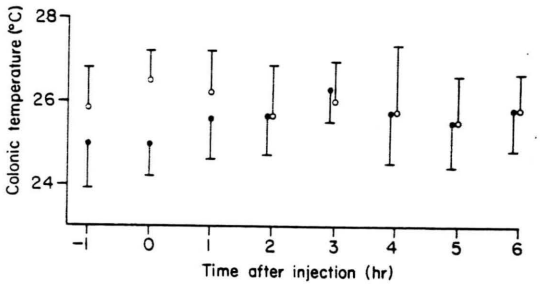

Fig. 5. Mean colonic temperature of *L. fuliginosus* ($n = 3$) on a day following injections of *S. typhosa* endotoxin (closed circles) and sterile pyrogen-free water (open circles). Injection at $t = 0$.

no pyrogen induced a colonic temperature significantly higher than that following saline injection, and *S. minnesota* indeed induced lower maximum colonic temperatures.

Figures 4 and 5 show the colonic temperature of *L. fuliginosus*, as a function of time, following injection of water and of LPS, on the day of injection and subsequent day. At no time was the colonic temperature of the snakes receiving LPS significantly different from that following water injection.

DISCUSSION

Given access to a heterogeneous thermal environment, the diurnal olive grass snake *P. phillipsii* regulated body temperature within a relatively narrow range around 33°C. Similar thermoregulatory competence has been observed in other reptiles, including snakes (De Witt, 1967; Johnson, 1972; Vaughn *et al.*, 1974). In some reptiles, this thermoregulatory competence has been ascribed not just to behavioural mechanisms but also to autonomic mechanisms (Chong *et al.*, 1973; Crawford and Kampe, 1971; Giordano and Jackson, 1973). The nocturnal brown house snake *L. fuliginosus* also regulated its body temperature, but even in the presence of a source of radiant heat, its temperature was significantly lower, namely about 25°C.

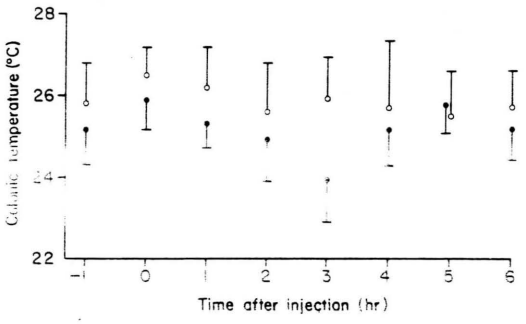

Fig. 4. Mean colonic temperature of *L. fuliginosus* ($n = 3$) following injections of *S. typhosa* endotoxin (10 μg/kg, closed circles) and equal volumes of sterile pyrogen-free water (open circles). Injection was at $t = 0$.

Although both snake species demonstrated the ability to control body temperature through behavioural thermoregulation, neither species developed fever, at least in response to the pyrogens we tested. In the diurnal snake *P. phillipsii*, which would have access to solar radiation in its normal environment, we tested endotoxin (the common pyrogenic moiety of all gram-negative bacteria), the specific gram-negative bacterium *S. minnesota*, and a gram-positive bacterium *S. aureus*. Whether we used maximum body temperatures or thermal response index as a measurement of fever, we could identify no evidence of fever in the snakes, whatever the pyrogen. We have shown recently that the leopard tortoise *Geochelone pardalis* also did not respond to the same pyrogens (Zurovsky *et al.*, 1987).

The nocturnal to crepuscular snake *L. fuliginosus* normally would have access to weak direct solar radiation and to warm substrates. In the experimental situation, we provided it with a warm substrate and with rather stronger direct radiation. In spite of the availability of adequate heat, the snake regulated its body temperature at about 25°C, a low preferred body temperature for reptiles (Spellerberg, 1982), but perhaps an appropriate one for a nocturnal reptile. In *L. fuliginosus* we tested only one pyrogen, namely endotoxin, and this pyrogen had no effect on body temperature. Since endotoxin accounts for all their pyrogenic activity, we conclude that no gram-negative bacterium would produce fever in the snake.

The failure of the snakes to develop fever to the traditional pyrogens was not the consequence of an inappropriate dose, or inactivity of the agent. In the same dose, all the pyrogens caused significant and long lasting fevers in New Zealand White rabbits.

We have therefore demonstrated that two members of another sub-order of reptiles, namely the Serpentes, did not elevate body temperature when challenged with pyrogens, even though their competence in behavioural thermoregulation would have enabled them to do so. These observations complement our previous results, which have shown that members of the sub-order Lacertilia (Laburn *et al.*, 1981), and one member of the Chelonia (Zurovsky *et*

al., 1987), also did not respond to the pyrogens. Our argument that fever is not ubiquitous, even among reptiles, is strengthened. Indeed, we believe that fever is rare amongst reptiles, and that the concept that it might have a general survival value which arose originally from observations of the fortuitous occurrence of fever in one family of lizards, is unsubstantiated.

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