J. Physiol. (1980), **303**. *pp.* 417–421
With* 1 *text-figure
Printed in Great Britain

FEVER AND ANTIPYRESIS IN THE CRAYFISH *CAMBARUS BARTONI*

BY M. E. CASTERLIN AND W. W. REYNOLDS
From the Center for the Life Sciences, University of New England,
Biddeford, Maine 04005, U.S.A.

(*Received 5 July 1979*)

SUMMARY

1. Normothermic *Cambarus bartoni* crayfish thermoregulating behaviourally in an electronic shuttlebox prefer a mean temperature of 22·1 °C ± 0·5 S.E. of mean.

2. Injection of 1 % saline, or addition of paracetamol to the ambient water, caused a statistically insignificant decrease in preferred temperature of 0·4 °C.

3. Injection of killed *Aeromonas hydrophila* bacteria into the abdomen caused a significant mean increase in preferred temperature of 1·8 °C (defined as a behavioural fever).

4. Injection of *A. hydrophila*, with paracetamol in the water, resulted in an insignificant increase of 0·1 °C above the normothermic temperature; thus paracetamol is antipyretic in crayfish, preventing fever but not altering afebrile thermoregulation.

5. These results suggest that similar mechanisms may be operating in the neuropharmacological mediation of fever in vertebrates and invertebrates.

INTRODUCTION

Fever in mammals is mediated by both autonomic and behavioural mechanisms (Clark & Lipton, 1974; Crawshaw & Stitt, 1975; Polk & Lipton, 1975; Satinoff, McEwen & Williams, 1976). Ectotherms (lower vertebrates and invertebrates) exhibit only rudimentary autonomic responses (Reynolds, 1977*a*; Nagai & Iriki, 1978; Reynolds & Casterlin, 1978) which are of little thermoregulatory significance (Reynolds, McCauley, Casterlin & Crawshaw, 1976; Reynolds & Casterlin, 1979), so that a fever cannot be produced autonomically. Ectotherms are capable, however, of behavioural thermoregulation (Reynolds & Casterlin 1979), and can become febrile through behavioural means, by seeking a higher than normal ambient temperature. The phenomenon of behavioural fever, defined as a significant increase in preferred temperature (Reynolds, Casterlin & Covert, 1976), has been observed in reptiles (Vaughn, Bernheim & Kluger, 1974), amphibians (Casterlin & Reynolds, 1977*a*), fishes (Reynolds *et al.* 1976), neonatal mammals (Satinoff *et al.* 1976) and arthropods (Casterlin & Reynolds, 1977*b*).

Behavioural fever can be induced in the crayfish *Cambarus bartoni* (Arthropoda: Crustacea: Decapoda) both by bacterial endotoxin (Casterlin & Reynolds, 1977*b*) and by prostaglandin E_1 (Casterlin & Reynolds, 1978). PGE_1 induces behaviourally

0022-3751/80/8220-0347 $07.50

and autonomically mediated fever in mammals (Crawshaw & Stitt, 1975), and various antipyretic drugs which block febrile responses in mammals are thought to do so by inhibiting prostaglandin synthesis (Vane, 1971; Ziel & Krupp, 1976), implying that endotoxin fever may be mediated by stimulation of prostaglandin synthesis.

As in mammals, the antipyretic paracetamol blocks the febrile response of fish (Reynolds, 1977*b*) to bacterial endotoxin. If prostaglandins play a role in the febrile response of arthropods, as they are inferred to do in vertebrates, antipyretics should block fever induction by endotoxin in arthropods. We report herein a test of this hypothesis with the crayfish *Cambarus bartoni*.

METHODS

Fifty *C. bartoni* were trapped in local streams and held in the laboratory at 20–25 °C until testing. The animals weighed 6–10 g each. They were individually tested in Ichthyotron electronic shuttleboxes described previously (Reynolds & Casterlin, 1976; Reynolds *et al.* 1976; Reynolds, 1977*c*), which permit an aquatic animal to control water and body temperatures automatically by means of normal unconditioned locomotor movements monitored by photocells. Temperatures and movements were recorded automatically without interference by the investigators. Two interconnected chambers, differing in temperature by 1–2 °C, heated or cooled in parallel according to movements of the animal as it sequentially interrupted paired light beams in passing between the two chambers. Because of the temperature differential between the chambers, operant conditioning of the animal is unnecessary; the animal simply reacts to temperatures as it would in nature: if it is too cool, it moves toward warmer temperatures, and if it is too warm, it moves toward cooler temperatures. When the animal moves from the cooler to the warmer chamber, parallel warming is initiated in both chambers; conversely, when the animal moves from the warmer to the cooler chamber, parallel cooling is initiated. So long as the animal remains in one chamber, warming or cooling (depending on which chamber the animal occupies) continues until the animal becomes too warm or too cool and is motivated to move to the other chamber. Thus the animal forms part of a feed-back loop by means of which it controls the water temperatures it experiences; and, because the body temperature of these small ectotherms is closely coupled to water temperature, the animal also controls its body temperature by its behaviour.

Each animal was allowed an initial 24 h period to habituate to the experimental apparatus and to gravitate to the species-specific final thermal preferendum (cf. Reynolds & Casterlin, 1979), previously established to be 22·1 °C for this species (Casterlin & Reynolds, 1977*b*). A further 24 hr then served as a normothermic control or base-line period. Then each animal was subjected to one of five different experimental treatments ($n = 10$ animals for each treatment group). One control group ($n = 10$) was untreated, and permitted to thermoregulate for a third 24 hr period in the shuttlebox. Animals in a second control group ($n = 10$) were injected with 0·1 ml. 1 % saline into the abdomen. For the third group ($n = 10$), one 325 mg paracetamol tablet, a standard size tablet dosage, was placed in each 50 l. chamber of the shuttlebox (cf. Green & Lomax, 1977). For the fourth group ($n = 10$), 0·1 ml. 1 % saline containing a suspension of 10^9 killed gram-negative bacterial cells (*Aeromonas hydrophila*) was injected into the abdomen. This bacterium had previously been found to be pyrogenic to *C. bartoni* (Casterlin & Reynolds, 1977*b*). Animals in the fifth group ($n = 10$) were similarly injected with *A. hydrophila*, but with paracetamol in the water as for group 3. For all five groups, thermoregulatory data were tabulated for comparison with data for the previous 24 hr control period. Use of 24 hr periods before and after treatment precluded any interference by circadian rhythm effects (Reynolds *et al.* 1976), as some aquatic ectotherms exhibit circadian rhythms of preferred temperature (cf. Reynolds, Casterlin, Matthey, Millington & Ostrowski, 1978).

RESULTS

The results are illustrated in Fig. 1. Untreated controls exhibited the same normothermic mean preferred temperature of 22·1 °C (± 0·5 s.e. of mean) during the third day as during the second day. Saline-injected controls exhibited no significant change in preferred temperature. The same was true for animals treated with paracetamol alone. The group injected with *A. hydrophila* alone became febrile, exhibiting a significant (*t* test, $P > 0{\cdot}01$) increase of 1·8 °C in preferred temperature; however, the final group, injected with bacteria in the presence of paracetamol, remained afebrile.

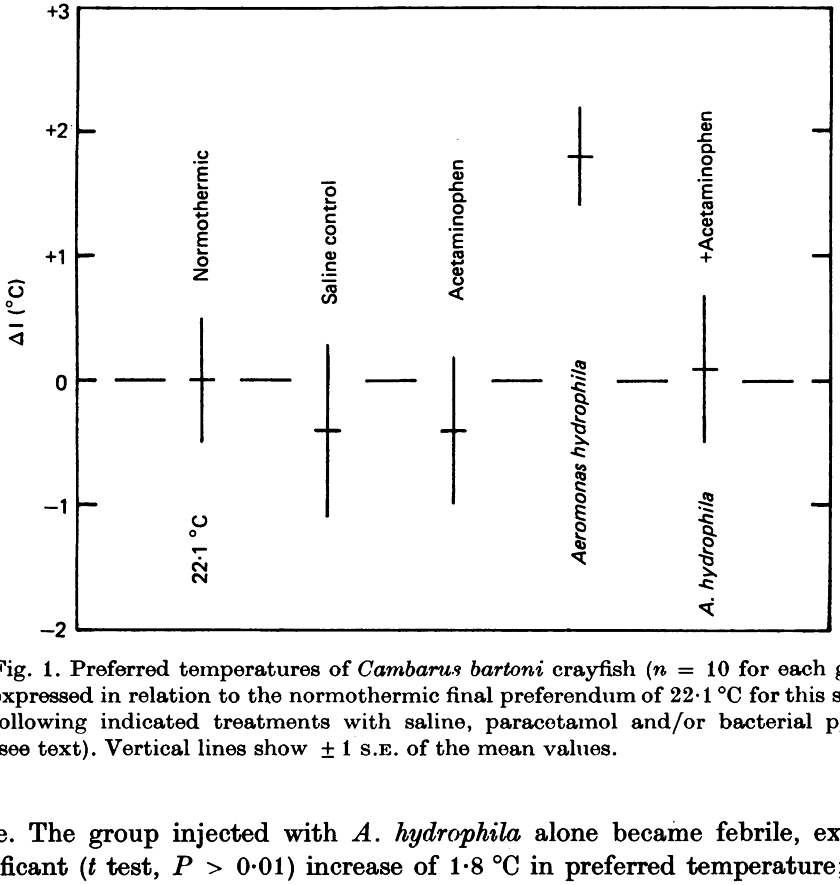

Fig. 1. Preferred temperatures of *Cambarus bartoni* crayfish ($n = 10$ for each group), expressed in relation to the normothermic final preferendum of 22·1 °C for this species, following indicated treatments with saline, paracetamol and/or bacterial pyrogen (see text). Vertical lines show ± 1 s.e. of the mean values.

DISCUSSION

The results clearly indicate that paracetamol is antipyretic in crayfish, blocking the normal pyrogenic action by which gram-negative bacteria such as *A. hydrophila* induce behavioural fever in *C. bartoni*. Paracetamol is presumably taken up through the gills, in crayfish as in fishes (cf. Reynolds, 1977*b*). Paracetamol alone does not significantly alter normothermic thermoregulation in crayfish, nor does the injection of saline.

We do not yet know the mechanism of antipyretic action of paracetamol in crayfish, but it is known that PGE_1 induces fever in *C. bartoni* (Casterlin & Reynolds, 1978), and antipyretics are thought to block febrile responses in mammals by interfering with prostaglandin synthesis (Vane, 1971; Ziel & Krupp, 1976). It therefore seems not unlikely that antipyretics may work in a similar fashion in arthropods, which would account for the results of our experiments. The evidence to date, therefore, suggests that febrile mechanisms in arthropods may be similar to those in vertebrates, which offers the possibility that these relatively simple invertebrates, which are easy and inexpensive to acquire and to maintain in the laboratory, may serve as convenient experimental models for further experimentation on the nature of thermoregulatory and febrile neuropharmacological mechanisms.

We are grateful for financial support from PSU Grants SAF-74-071, SAF-77-113 and SAF-78-050, and from NSF Grant PCM-78-05691, to W.W.R. We thank Dr John Bligh for comments upon the manuscript.

REFERENCES

CASTERLIN, M. E. & REYNOLDS, W. W. (1977*a*). Behavioural fever in anuran amphibian larvae. *Life Sci., Oxford* **20**, 593–596.

CASTERLIN, M. E. & REYNOLDS, W. W. (1977*b*). Behavioral fever in crayfish. *Hydrobiologia* **56**, 99–101.

CASTERLIN, M. E. & REYNOLDS, W. W. (1978). Prostaglandin E_1 fever in the crayfish *Cambarus bartoni*. *Pharmacol. Biochem. & Behav.* **9**, 593–595.

CLARK, W. G. & LIPTON, J. M. (1974). Complementary lowering of the behavioural and physiological thermoregulatory set-points by tetrodotoxin and saxitoxin in the cat. *J. Physiol.* **238**, 181–191.

CRAWSHAW, L. I. & STITT, J. T. (1975). Behavioural and autonomic induction of prostaglandin E_1 fever in squirrel monkeys. *J. Physiol.* **244**, 197–206.

GREEN, M. D. & LOMAX, P. (1977). Behavioural thermoregulation and neuroamines in fish (*Chromus chromus*). *J. thermal Biol.* **1**, 237–240.

NAGAI, M. & IRIKI, M. (1978). Autonomic response of the fish to pyrogen. *Experientia* **34**, 1177–1178.

POLK, D. L. & LIPTON, J. M. (1975). Effects of sodium salicylate, aminopyrine and chlorpormazine on behavioral temperature regulation. *Pharmacol. Biochem. & Behav.* **3**, 167–172.

REYNOLDS, W. W. (1977*a*). Thermal equilibration rates in relation to heartbeat and ventilatory frequencies in largemouth blackbass, *Micropterus salmoides*. *Comp. Biochem. Physiol.* **56**A, 195–201.

REYNOLDS, W. W. (1977*b*). Fever and antipyresis in the bluegill sunfish, *Lepomis macrochirus*. *Comp. Biochem. Physiol.* **57**C, 165–167.

REYNOLDS, W. W. (1977*c*). Fish orientation behavior: an electronic device for studying simultaneous responses to two variables. *J. Fish. Res. Bd Can.* **34**, 300–304.

REYNOLDS, W. W. & CASTERLIN, M. E. (1976). Thermal preferenda and behavioral thermoregulation in three centrarchid fishes. In *Thermal Ecology II*, ed. ESCH, G. W. & MCFARLANE, R. W., pp. 185–190. Springfield, Va.: U.S. Natl. Tech. Info. Serv.

REYNOLDS, W. W. & CASTERLIN, M. E. (1978). Estimation of cardiac output and stroke volume from thermal equilibration and heartbeat rates in fish. *Hydrobiologia* **57**, 49–52.

REYNOLDS, W. W. & CASTERLIN, M. E. (1979). Behavioral thermoregulation and the 'final preferendum' paradigm. *Am. Zool.* **19**, 211–224.

REYNOLDS, W. W., CASTERLIN, M. E. & COVERT, J. B. (1976). Behavioural fever in teleost fishes. *Nature, Lond.* **259**, 41–42.

REYNOLDS, W. W., CASTERLIN, M. E., MATTHEY, J. K., MILLINGTON, S. T. & OSTROWSKI, A. C. (1978). Diel patterns of preferred temperature and locomotor activity in the goldfish *Carrassius auratus*. *Comp. Biochem. Physiol.* **59**A, 225–227.

REYNOLDS, W. W., MCCAULEY, R. W., CASTERLIN, M. E. & CRAWSHAW, L. I. (1976). Body temperatures of behaviorally thermoregulating largemouth blackbass (*Micropterus salmoides*). *Comp. Biochem. Physiol.* **54**A, 461–463.

SATINOFF, E., MCEWEN, G. N. JR. & WILLIAMS, B. A. (1976). Behavioral fever in newborn rabbits. *Science, N.Y.* **193**, 1139–1140.

VANE, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, Lond.* **231**, 232–235.

VAUGHN, L. K., BERNHEIM, H. A. & KLUGER, M. J. (1974). Fever in the lizard *Dipsosaurus dorsalis*. *Nature, Lond.* **252**, 473–474.

ZIEL, R. & KRUPP, P. (1976). Mechanisms of action of antipyretic drugs. In *Brain Dysfunction in Infantile Febrile Convulsions*, ed. BRAZIER, M. A. B. & COCEANI, F., pp. 141–152. New York: Raven.