



FEVER AND METABOLIC RATE IN THE TOAD *BUFO MARINUS*

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Abstract—1. Toads injected with the pyrogen LPS had a higher metabolic rate than control toads injected with saline if the toads were held at the febrile temperature of 32°C, but not if they were held at the normothermic temperature of 25°C.

2. We suggest that the elevated metabolic rate of febrile toads reflects the cost of the stimulation of the immune system.

3. We discuss the evolutionary implications of these data, and suggest that the febrile response of vertebrate ectotherms and endotherms is homologous. © 1998 Elsevier Science Ltd. All rights reserved

Key Word Index: Fever; metabolic rate; oxygen consumption; toad, amphibian; ectotherm

INTRODUCTION

Fever has been well documented among both vertebrates and invertebrates and appears to have a long phylogenetic history (Cabanac, 1990; Kluger, 1991). Among mammals and birds, fever is established by an increase in metabolic heat production and the physiological regulation of the body temperature at a higher set point. Among ectotherms, fever is manifest as a higher selected environmental temperature which confers a higher body temperature (Bernheim and Kluger, 1976; Sherman *et al.*, 1991).

In mammals, the increase in metabolic heat production of fever is associated with enhanced immunologic function (Kluger, 1986). Given that ectotherms fight infections more effectively when permitted to acquire behavioural fever (Kluger *et al.*, 1975; Covert and Reynolds, 1977; Boorstein and Ewald, 1987), it is reasonable to predict that elevated metabolism would be a physiological correlate of fever in ectotherms as well. Using a treatment that induced behavioural fever in reptiles in an earlier study (Kluger, 1978), Malvin and Kluger (1979) found no measurable increase in oxygen consumption among green iguanas injected with dead bacteria. However, they only tested the iguanas at normothermic temperatures and did not permit the animals to acquire a behavioural fever. Sherman *et al.* (1991) induced behavioural fevers in the toad, *Bufo marinus*

by injecting them with the pyrogen lipopolysaccharide (LPS). This was associated with an increase in the thermal tolerance of injected toads only when the animals were permitted to become febrile, but not when they were held at normothermic temperatures. Similarly, increases in metabolic rate may only be manifest when pyrogen treated ectotherms are maintained at febrile temperatures.

In this study, we examined the metabolic rate of pyrogen-injected toads held at normothermic temperatures and febrile temperatures. Given the long evolutionary history of fever and the metabolic changes that underlie this defense response to infection in endotherms, we suggest that there are homologous metabolic changes that accompany fever in ectotherms.

MATERIALS AND METHODS

Male and female toads (132–237 g), *Bufo marinus*, were obtained from a commercial supplier and maintained in aquaria with access to both free water and dry areas at 21.0 (± 2.0)°C on a 12:12 light–dark (L:D) photoperiod (centred at noon EST). The toads were fed live crickets twice a week, but were kept unfed for a minimum of 15 h prior to treatment.

Treatment

We used the same procedure that induced behavioural fever in *Bufo marinus* in an earlier study from this laboratory (Sherman *et al.*, 1991). The pyrogen, lipopolysaccharide (LPS) from *Escherichia*

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Table 1. Analysis of variance to examine the contributions of injection, temperature, and mass, on metabolic rate in toads. Injection treatment and temperature class were used as categorical variables and mass as a covariate in the ANOVA model

Source	Degrees of freedom	Mean square	F-Ratio	P
Inject	1	8.244	20.575	<0.001
Temp	1	13.164	32.855	<0.001
Inject*Temp	1	4.08	10.183	0.004
Inject*Mass	1	0.444	1.108	0.304
Error	21	0.401		

coli (Serotype 0127:B8) was obtained from Sigma Chemical Co. and prepared under sterile conditions at a concentration of 15 mg/ml of 0.7% saline (made using sterile, pyrogen-free water). Pyrogen-treated toads received a subcutaneous injection of 1 mg LPS/50 g wet mass and control toads were injected with a comparable volume of saline. Following injection at 0900 h EST, toads were incubated in the dark in a constant temperature cabinet at either the normothermic temperature of 25.0 (± 1.0)°C or the febrile temperature of 32.0 (± 1.0)°C (see Sherman *et al.*, 1991). Each toad was incubated for 7 h in a 4 l container lined with moist paper towel.

Oxygen consumption

After the 7 h incubation period, the oxygen consumption of each toad was measured in the dark in a humid container at the toad's incubation temperature (25 or 32°C) using an open flow system following the methods of Longphre and Gatten, 1994 for differential oxygen concentration. Air streams from the animal container and a similar empty container were drawn through tubes of drierite (to remove water vapour) and ascarite (to remove carbon dioxide) using an Ametek Flow Control R-2 flowmeter. The two air streams were then directed through an Ametek Model S-3AII oxygen analyzer (at a rate of 70 ml min⁻¹) and differential oxygen concentration readings were recorded every 15 minutes for 2 h. Only the last five readings were used in calculations of mean oxygen consumption rates in order to minimize the effects of handling on the metabolic rates of the toads. Thus, the data used were collected 9 h after injection which was well within the period during which LPS-injected toads express fever (Sherman *et al.*, 1991). Oxygen consumption rates are reported in ml O₂ h⁻¹ at STPD and were calculated using standard equations (Gatten *et al.*, 1992).

From 5 to 8 toads were used in each of the four treatment groups (saline-25°C, LPS-25°C, saline-32°C, LPS-32°C) and each toad was used only once. All animals recovered from the procedure.

Statistical analyses. We used ANOVA to define the contribution of toad mass to the variation in metabolic rate. Injection treatment (LPS or saline) and temperature class were used as categorical variables and mass as a covariate in the ANOVA model. The differences in mean oxygen consumption rates of saline and LPS-injected toads within one temperature treatment were analyzed using Student's *t*-test.

RESULTS

We examined the metabolic rates of control toads (saline-injected) at 25°C (normothermic) and 32°C (febrile) and compared them to the metabolic rates of LPS-injected toads at those same temperatures. In the ANOVA using injection treatment and temperature as categorical independent variables and mass as a covariate, the variation in metabolic rate explained by mass was not significant (Table 1, $F = 1.108$; $P = 0.304$) and thus, we pooled data from toads within each treatment group. Injection treatment, temperature and their interaction were highly significant (Table 1).

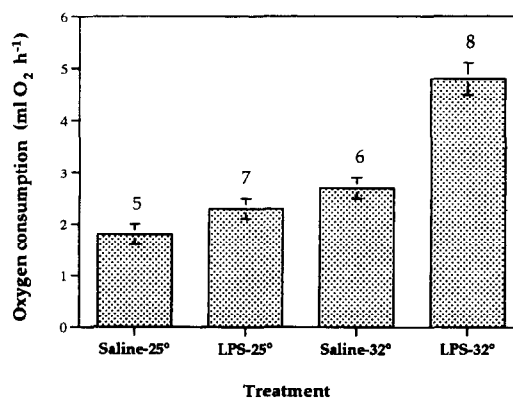


Fig. 1. Mean oxygen consumption (oxygen consumed in ml h⁻¹; \pm SEM) of toads in the four different treatment groups: two groups of control toads (saline-injected at 25°C and saline-injected at 32°C) and two groups of treated toads (LPS-injected at 25°C and LPS-injected at 32°C). Numbers indicate sample sizes of the different groups.

Mean metabolic rates (O_2 consumed in $ml\ h^{-1}$) of toads from the four different treatment groups are compared in Fig. 1. The mean rate of oxygen consumption of LPS-injected toads at $25^\circ C$ ($2.3 \pm 0.2\ ml\ O_2\ h^{-1}$) was greater than that of saline-injected control toads at $25^\circ C$ ($1.8 \pm 0.2\ ml\ O_2\ h^{-1}$), but the difference was not significant ($0.05 < P < 0.1$). However, when the animals were incubated at the febrile temperature of $32^\circ C$, the mean oxygen consumption rate of LPS-injected toads ($4.8 \pm 0.3\ ml\ O_2\ h^{-1}$) was significantly greater than that of saline-injected toads ($2.7 \pm 0.2\ ml\ O_2\ h^{-1}$) ($P < 0.005$).

DISCUSSION

To our knowledge, this is the first study to demonstrate an increase in metabolic rate following pyrogen injection in an ectotherm. While the small increase in metabolic rate between control and LPS-injected toads held at the normothermic temperature of $25^\circ C$ approached significance, a substantial increase in oxygen consumption rate between saline-injected and LPS-injected toads was observed only if animals were held at the febrile temperature of $32^\circ C$. Malvin and Kluger (1979) also failed to find an increase in oxygen consumption rate in bacteria-injected lizards that were held at a non-febrile temperature. However, they did not test their animals at the febrile temperature. Similarly, Sherman *et al.* (1991) found that the thermal tolerance of LPS-injected toads increased only if the toads were permitted to become febrile but not if they were held at the normothermic temperature.

The increase in metabolic rate demonstrated in this study is not simply a passive consequence of an elevated body temperature, because LPS-injected toads held at $32^\circ C$ had a higher metabolic rate than saline-injected toads at $32^\circ C$. Rather, the increase may reflect the cost of immuno-stimulation. The myriad of physiological components underlying the immune response are discussed in detail elsewhere (Kluger, 1991), but include increased activity of white blood cells and, in particular, activation of T lymphocytes. The metabolic costs of such processes are unknown, but deserve further study. It might be difficult to partition the increase in metabolic rate observed in febrile endotherms into its elevated temperature and immuno-stimulation components. It may well be simpler to ask such questions using ectotherms.

The rates of oxygen consumption in *Bufo marinus* we report here are similar to rates reported by others (see Gatten *et al.*, 1992). The temperature coefficient or Q_{10} of most chemical reactions is between 2 and 3. In our study, the Q_{10} of oxygen

consumption rates for saline-injected toads between 25 and $32^\circ C$ was 1.8. The Q_{10} of oxygen consumption rates for the control toads at $25^\circ C$ compared to LPS-injected toads at the febrile temperature of $32^\circ C$ was 4.1 and reflects the high energy expenditure of febrile ectotherms.

The results of the present study further support the claim that the febrile response to infection has a long evolutionary history (Cabanac, 1990; Kluger, 1991). It is likely that the behavioural fever expressed by infected ectothermic vertebrates is homologous to the physiological fever of infected birds and mammals. Infected ectotherms permitted to become febrile have higher rates of survival (Kluger *et al.*, 1975; Covert and Reynolds, 1977; Kluger, 1986), express concomitant physiological changes that enable them to endure higher temperatures (Sherman *et al.*, 1991) and, based on the results of the present study, generate higher metabolic rates, perhaps in support of the enhanced immune response accompanying fever. In spite of its metabolic costs, this response appears to be highly conserved and likely has adaptive significance (Kluger, 1991). We would predict that infected vertebrate ectotherms would express enhanced immunologic function if permitted to become febrile.

In addition to vertebrates, arthropods, annelids, and mollusks express behavioural fever as well (Cabanac, 1990). Is the febrile response of vertebrates and these protostome invertebrates homologous or did fever evolve more than once? Cabanac and Drolet (1991) found no evidence of the febrile response in planaria following injection with several agents which induce fevers in other animals. Nevertheless, negative results may be due to the choice of methodologies (inappropriate agents, inappropriate doses, etc.) and should not be taken to mean the absence of the effect altogether. Further study of the response to infection in pre-coelomate animals would shed light on the evolutionary history of fever.

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