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M. BODURKA[1], M. CAPUTA[2], J. BODURKA[3]

A COMPARISON OF FEBRILE RESPONSES INDUCED BY LPS FROM *E. COLI* AND *S. ABORTUS* IN UNRESTRAINED RATS PLACED IN A THERMAL GRADIENT.

Department of Physiology[1], Department of Biophysics[3] University School of Medical Sciences, Bydgoszcz
Department of Animal Physiology[2], Institute of Biology, N.Copernicus University, Toruń, Poland.

The purpose of our study was a comparison of pyrogenic and behavioural effects of *Escherichia coli* (*E. coli*) and *Salmonella abortus* (*S. abortus*) endotoxins in unrestrained, freely moving Wistar rats, placed in a thermal gradient and having an easy access to ambient temperatures within a range 5—40 °C. Hypothalamic and chosen by the rats ambient temperatures as well as locomotor activity were recorded before and after intraperitoneal injection of 1 mg/kg lipopolysaccharide from *E. coli* or from *S. abortus*. Control animals were injected with pyrogen-free saline.Both endotoxins induced warm-seeking behaviour which was accompanied by biphasic fever. Locomotor activity of LPS-injected rats was reduced. *S. abortus*-induced fever peaked at 100th minute (reaching 37.5 ± 0.2° C) and at 250th minute (reaching 38.1 ± 0.1°C). Respective data for *E. coli* fever were: 170th minute (when hypothalamic temperature reached 37.6 ± 0.3°C) and 430th minute (with hypothalamic temperature of 38.6 ± 0.1°C). Comparing to *S. abortus*-generated fever both peaks of *E. coli* LPS-induced fever were significantly delayed ($p < 0.05$). A limited structural variability of lipid A from both bacteria is likely to be responsible for the difference in fever timing recorded in this study.

Key words: *rats, behavioural thermoregulation, locomotor activity, thermal preference, fever.*

INTRODUCTION

Lipopolysaccharide (LPS) is an integral component of the membrane of gram-negative bacteria such as *Escherichia coli* (*E. coli*) and *Salmonella abortus* (*S. abortus*) (1). It has been known that after injection of LPS many mammalian species show a variety of responses which include mainly the febrile elevation of body temperature, hypersomnia, depressed activity, loss of interest in social contacts and depressed food intake. These changes are termed "sickness behaviour". Such a system of behavioural responses is an important

component of the host defence against infection designed to maintain homeostasis (2). The pyrogenic properties of LPS (endotoxin) are dependent on stimulating numerous immune cells of the host to release endogenous pyrogens mainly Il-1β and TNF (3). Some data (4,5) support the hypothesis that Il-1β induces a rise in hypothalamic Il-1β that triggers a rise in prostaglandins (particularly PGE_2) in this area (6). These can directly modify activity of thermosensitive neurones in the preoptic anterior hypothalamus (POAH), resulting in the febrile elevation of body temperature (3). However, there are some data that injection of LPS in the rats does not result in a fever but rather in a fall of body temperature (7). That has been explained by a marked heat loss due to a large relative surface area in small mammals such as rodents (8). Moreover, it has recently been found that adult, unrestrained mice who do not show pyrogenic reaction to LPS at room temperature can augment their body temperature by choosing a higher ambient temperature in a temperature gradient (9, 10). *E. coli* is always present in the normal intestinal flora of animals. On the other hand, *S. abortus* is a pathogen which causes the gastroenteritis (11). Because the ecological niches and characteristics of these bacteria are different it is likely that they induce also different febrile responses.

The purpose of this study was to compare the action of *E. coli* and *S. abortus* endotoxins on hypothalamic temperature (T_h), ambient temperature chosen by animals (T_a) and locomotor activity in unrestrained rats placed in a thermal gradient system.

MATERIALS AND METHODS

Animals

Altogether ten adult (six months old) male Wistar rats were used. Animals were kept at a room temperature and were exposed to seasonal illumination conditions. Water and commercial chow pellets were available ad libitum.

Surgery

One to two weeks prior to experiments animals were anaesthetized with ketamine (130 mg/kg, i.p). Hypothalamic thermocouple guide tubes were implanted into the preoptic area of the hypothalamus according to the atlas of Paxinos and Watson (12). The position of the sensors was verified post-mortem.

Equipment

Ambient temperature and locomotor activity were recorded using the temperature gradient system (13) controlled by two PC computers. It was an aluminium tube, 2 m long, open at its upper part, heated at one end and cooled at the other. Overall range of temperature along the tube varied from 5°C to 40°C. There was a series of thermoelements connected in pairs to infrared sensors along the gradient. These served to compute the position of the animal and simultaneously to record ambient temperature selected by the rats. Computer software was designed to calculate the change in position of the rat and thereby to provide a measure of its locomotor activity.

Hypothalamic-implanted rats were connected to a computer controlled recording system with a thermocouple made of 0.1 mm copper and constantan wires. The small voltage from thermocouples was amplified by the specialised PCLD 779 card (Advantech) and then was converted to digital signal by the PCL 711B card (Advantech). Finally, the hypothalamic temperature was calculated by controlling software.

Experimental procedure

The animals were placed in the temperature gradient system and their locomotor activity as well as hypothalamic and selected ambient temperatures were recorded. Endotoxins or saline were administred after the steady state of hypothalamic temperature had been monitored for at least 30 minutes (usually between 8.00 and 9.00 hr). The first group of rats was injected intraperitoneally with LPS from *Escherichia coli 0111:B4* (Sigma) in the dose of 1 mg/kg dissolved in 1 ml of saline. The second group was given the same dose of LPS from *Salmonella abortus equi* (Sigma). Control animals were injected with the same volume of pyrogen-free saline. Experiments were continued until fever responses ceased.

Statistics

The data were expressed as means ± standard deviations (SDs). Differences in responses to *E. coli* and *S. abortus* endotoxins were evaluated using the unpaired Student's t-test.

RESULTS

A comparison of fevers induced by LPS from S. abortus and E. coli

Each injection, both with saline and endotoxin, induced immediate increase in hypothalamic temperature which disappeared after 20 – 30 minutes (*Fig. 1*). In saline-injected rats that was followed by frequent oscillations in the cerebral temperature due to behavioural cycles. On the other hand, rats injected with both endotoxins showed biphasic fevers. After *S. abortus* endotoxin (*Fig. 1*, left panel) rats reached a first peak of fever (37.5 ± 0.2°C) 100 minutes after injection and a second peak (38.1 ± 0.1°C) 250 minutes after the treatment. Six hours after injection the T_h returned to a normal level of 37.1 ± 0.1°C.

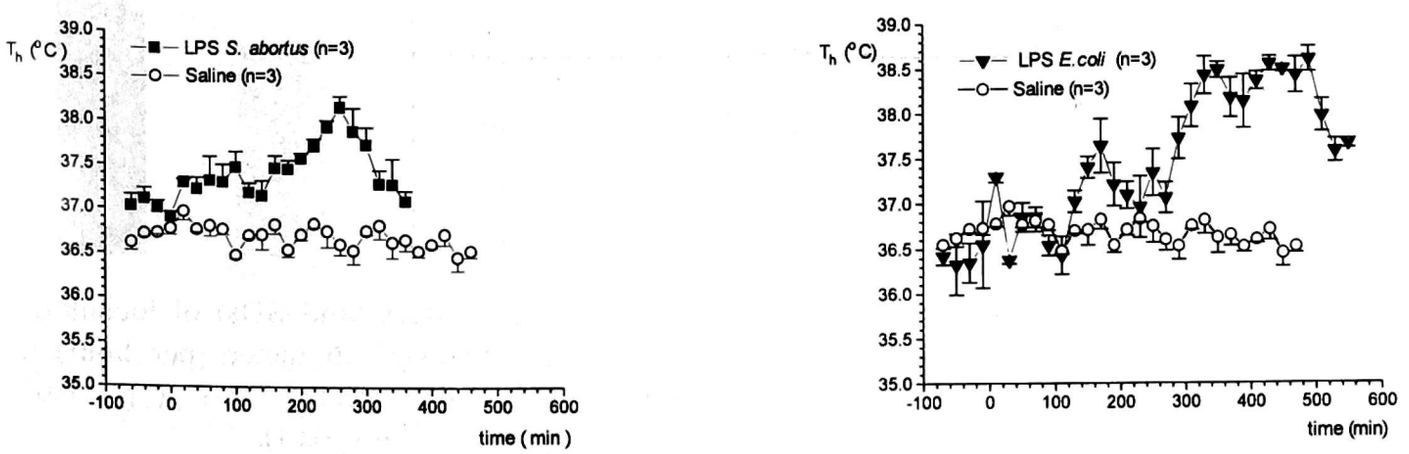

Fig. 1. Time courses of mean (with SDs) hypothalamic temperatures (T_h) in LPS *S. abortus* (left panel) and LPS *E. coli* (right panel) injected rats. LPS and saline (in control experiments) were injected at time 0.

Rats treated with *E. coli* endotoxin (*Fig. 1*, right panel) reached a first peak of fever (37.6 ± 0.3°C) 170 minutes following injection and a second peak (38.6 ± 0.1°C) 430 minutes after the treatment, and then cerebral temperature started to decrease. Both peaks of fever in rats injected with *E. coli* endotoxin were significantly ($p < 0.05$) retarded as compared to *S. abortus* fever.

A comparison of selected ambient temperature and locomotor activity in rats treated with S. abortus and E. coli endotoxins

Figure 2 shows changes in selected temperature during both bouts of fever induced by *S. abortus* and *E. coli* endotoxins. Both phases of fever generated by both endotoxins were preceded by increases in ambient temperature selected by rats. However, there was a difference in responses to the two kinds of endotoxins (*Fig. 2*).

Both endotoxins decreased motor activity of rats (*Fig. 3*), although the change was more pronounced after *S. abortus* endotoxin and it was highly significant as compared to control animals ($p < 0.01$).

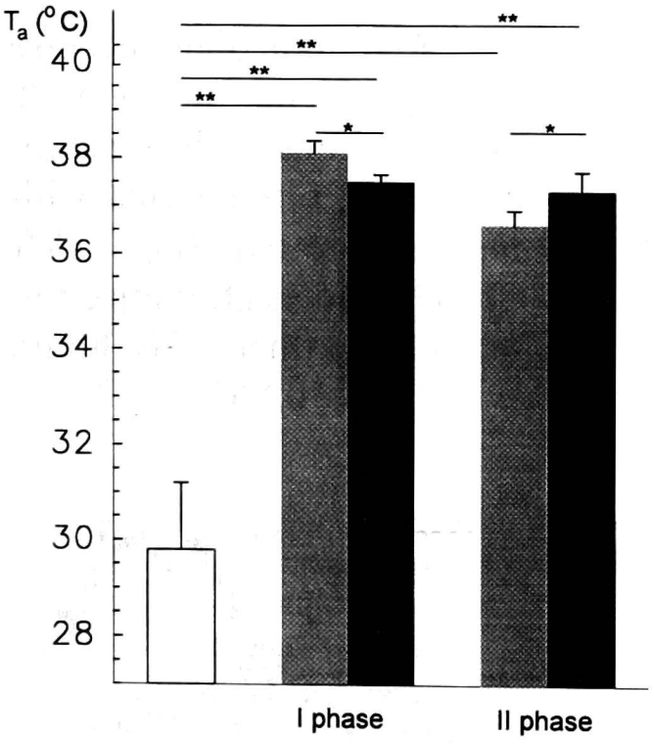

Fig. 2. Mean values (and SDs) of selected ambient temperature after saline (empty bar), LPS *S. abortus* (grey bar) and LPS *E. coli* (black bar) injections. Values for LPS were collected from a period of the first (I) and the second (II) phase of fever (*$p < 0.05$; **$p < 0.01$).

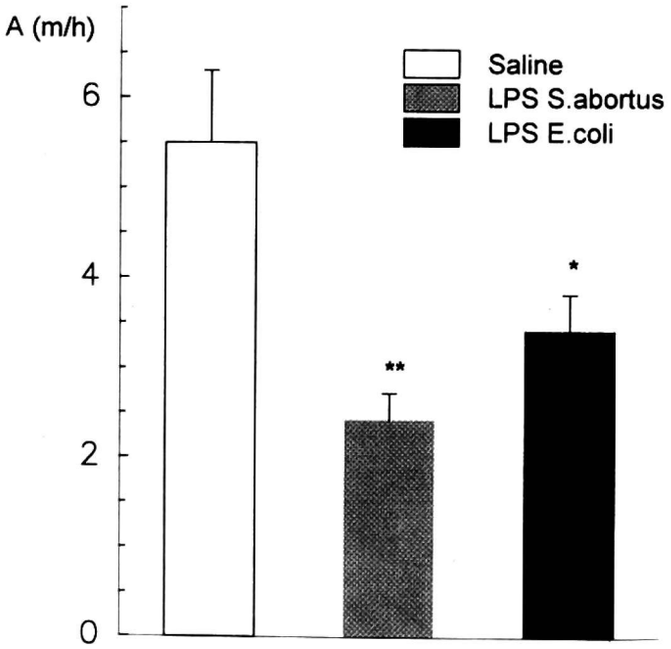

Fig. 3. Mean values (and SDs) of locomotor activity A (expressed in meters per hour) in saline and LPS treated rats (*$p < 0.05$; **$p < 0.01$).

An atypical response to E. coli endotoxin

One of our experiments resulted in an abnormal response to *E. coli* endotoxin (*Fig. 4*). There was a prompt increase in cerebral temperature to 37.8°C at 40th minute the postinjection period and then T_h exhibited a rapid fall down to 34.4°C one hour later. That hypothermic response was preceded by a decrease of the selected temperature down to 21.4°C.

Because such a hypothermic response was an exception in our study we decided not to use it for statistical analysis.

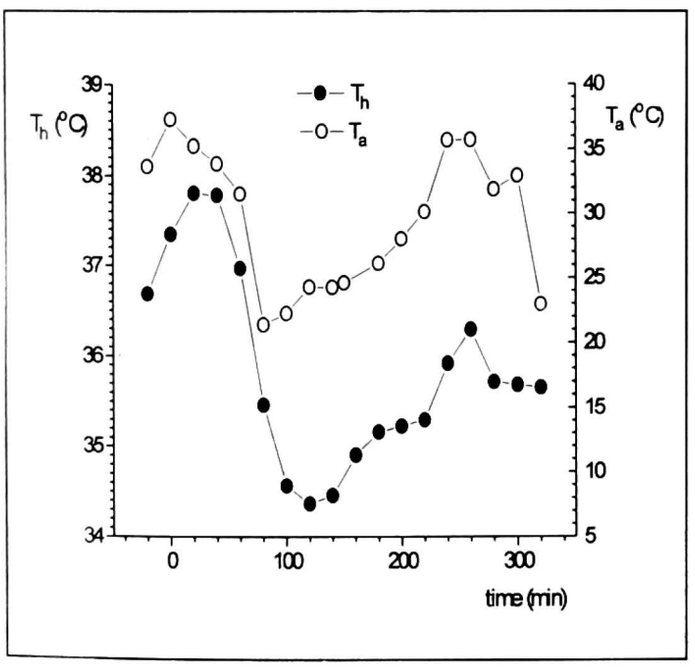

Fig. 4. Time courses of hypothalamic temperature (T_h; filled circles) and selected ambient temperature (T_a; empty circles) in a rat showing hypotermic response to LPS *E. coli* injection.

DISCUSSION

The maintenance of body temperature within a narrow range in homeotherms is under direct control of POAH, which modifies peripheral effector mechanisms of heat production and heat loss. Variations in heat loss can be achieved by both autonomic (changes in local blood flow, piloerection, sweating) and behavioural (huddling, licking) processes. Variations in heat production are largely under autonomic control and depend on either shivering or non-shivering thermogenesis. The latter predominates in small mammals (14, 15). Our results indicate that unrestrained and freely moving rats placed in the thermal gradient show a normal cerebral temperature of 36.4 ± 0.2 °C and they select ambient temperature of 29.8 ± 1.4°C. This confirms the estimation of Poole *et al.* (16) that in rats zone of minimal metabolic rate is between 28 and 32°C. In this range of T_a rats exhibit low motor activity, which apparently is a contributing factor of the minimal metabolic rate (16). The selected T_a is associated with minimal thermal conductance which indicates relatively low peripheral blood flow (17).

Fever is defined as a regulated increase in body temperature (15). Development of fever is achieved by a reduction in heat loss and increased heat production. When rats treated with both *E. coli* and *S. abortus* endotoxins developed a febrile elevation of cerebral temperature they simultaneously selected higher T_a. Such results are in agreement with hypothesis that in small rodents placed in a thermoneutral or warm environment fever is accomplished by the inhibition of heat loss rather than by an increase of heat production (18). Moreover, some studies suggest that in small mammals behavioural thermoregulatory mechanisms are activated before autonomic reflexes (19).

The responses evoked by LPS have been shown to involve a number of brain regions and neurotransmitter systems (20). However, when LPS is given as an intraperitoneal injection, the infection is localised (e.g. peritonitis and gastritis) and occurs far from the central nervous system. The classical explanation for such a discrepancy is that the message is transmitted humorally (21). Peripheral immune stimuli induce the expression of pro-inflammatory cytokines in the brain (22). However , some recent studies suggest that signals influencing sickness behaviour in rats can be transmitted via the vagus nerve (23).

Proinflammatory cytokines have potent activating effects on the hypothalamic-pituitary-adrenal axis which account for the increase in plasma glucocorticoids that, in turn occurs during the response of the host to infection (24). In the case of Il-β induced by LPS, this effect appears to be mediated at the hypothalamic levels and involves an enhanced release of coriticotropin releasing hormone (25) and noradrenaline (26). On the other hand, glucocorticoids feedback on activated immune cells to downregulate the synthesis and release proinflamatory cytokines. In the absence of such a regulatory feedback loop animals are much more sensitive to infection (27). The transient increase of cerebral temperature after a few minutes of exposure to stressful conditions (induced by handling and injection) may be due to an enhancement, stress-induced Il-6 secretion which is probably mediated by a glucocorticoid-independent mechanism (28). Some data (29) show that TNFα plays a complex role in the mechanism of fever and support the hypothesis that this cytokine is involved in endogenous system that lowers body temperature or that limits the magnitude of fever. An unexpected fall in cerebral temperature which we observed in one animal treated with *E. coli* endotoxin can be explained by stimulation of TNF production. Such a hypothermic response, however, is typical of rats injected intraperitoneally with LPS doses as high as 5 mg/kg (Caputa *et al.*, to be published). On the other hand, intravenous treatment of rats with *E. coli* LPS induces a body temperature fall already at 0.5 mg/kg (30). Therefore, it is likely that the abnormal response that we could observe in this study was due to unintentional intravenous administration of endotoxin as a consequence of accidental puncture of

a visceral vessel. Also timing of the hypothermic response in our experiment is indicative of intravenous route of injection - it was two hours postinjection which coincides with the response of intravenously injected rats (30).

Effects of both *E. coli* and *S. abortus* endotoxins are the result of interaction of lipid A with mononuclear host cells. These cells in turn secrete endogenous mediators such as cytokines and metabolites of arachidonic acid (prostaglandins) and these mediators act in different body organs and brain regions (1). A limited structural variability of lipid A from both bacteria may explain some differences in the latency and in the time course of fevers due to initiating sooner or later the behavioural responses and autonomic reflexes which adjust body temperature to the elevated thermoregulatory set point.

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Autor's address: M. Bodurka, Department of Physiology, University School of Medical Sciences, 24 Karłowicza Str. 85-092 Bydgoszcz, Poland.
E-mail: BODURKA at ACI. AMB. BYDGOSZCZ, PL