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Ambient temperature modulates the magnitude of LPS-induced fevers in Pekin ducks

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ABSTRACT

The consequences of variations in environmental temperature on innate immune responses in birds are by and large not known. We investigated the influence of ambient temperature on the febrile response in female Pekin ducks (*Anas platyrhynchos*). Ducks, implanted with temperature data loggers to measure body temperature, were injected with lipopolysaccharide (100 μg kg^{-1}) to evoke febrile responses and kept at ambient temperatures higher, within, and lower than their thermoneutral zone ($n=10$), and in conditions that simulated one day of a heat wave ($n=6$). Compared to the febrile response at thermoneutrality, at low temperatures, febrile responses were significantly attenuated; fevers reached lower magnitudes (from basal body temperature of 41.2 ± 0.3 °C to a peak of 42.0 ± 0.3 °C). In contrast, at high ambient temperatures, ducks rapidly developed significantly enhanced fevers, which reached markedly higher febrile peaks (from basal body temperature of 41.6 °C to a peak of 44.0 °C in a simulated heat wave when ambient temperature reached 40 °C). These results indicate that ambient temperature affects the febrile response in female Pekin ducks. Our findings reveal a key difference in febrile mediation between ducks and mammals, and have implications for avian survival because high environmental temperatures during febrile mediation could lead to febrile responses becoming physiologically deleterious.

1. Introduction

A rise in ambient temperature and the expected increase in new emerging pathogens, as a result of climate change, pose novel demands on the physiology of homeothermic animals (La Sorte and Jetz, 2010; McCarthy, 2001; Thomas et al., 2004). Knowledge regarding the impact of high ambient temperatures on the innate immune system in birds, would improve our understanding of how avian species might cope with changing environments and would provide information regarding the energy requirements of immune system activation in diverse climates.

Fever is considered an ancient defensive response employed by a host's innate immune system in response to infection (Ewald, 1980; Hart, 1988; Kluger et al., 1996). Reptiles and amphibians, the evolutionary ancestors of modern day mammals and birds, raise their body temperature by seeking hot environments after exposure to bacterial endotoxin (Bronstein and Conner, 1984; Casterlin and Reynolds, 1977; Ewald, 1980; Reynolds et al., 1976). This behavioral fever decreases the mortality due to infection (Bernheim and Kluger, 1976; Ewald, 1980). In homeotherms, fever is augmented by systemic physiological responses, including vasomotor adjustments and elevated heat production (Kluger et al., 1996). The physiological mechanisms responsible for fever are comparable in mammals and some bird species (Gray et al., 2005; Romanovsky et al., 2005). For instance, the fever response to bacterial endotoxin in ducks, pigeons and chickens as well as in all mammals is mediated by the pro-inflammatory cytokines interleukin (IL)-6 and IL-1β, and also by prostaglandins and nitric oxide, as well as being modulated by glucocorticoids (Cartmell et al., 2000; Gray et al., 2005; Johnson et al., 1993b; Klasing, 1998; Macari et al., 1993; Nakamura et al., 1998; Romanovsky et al., 2005; Roth, 2006).

The influence of ambient temperature on the mammalian febrile response has been well studied over the past 5 decades, but considerably less is known about the influence of ambient temperature on the avian febrile response. The febrile response in most mammals is independent of ambient temperature (Crawshaw and Stitt, 1975; Hasday et al., 2000; Roberts, 1991; Shoham and Krueger, 1988; Stitt, 1973; Tegowska et al., 1986; Won and Lin, 1983) although rats and mice kept in cool environments, and injected with pyrogen, develop hypothermia before the onset of fever (rats) (Romanovsky et al., 2002) or after the second febrile

Abbreviations: DTRI, differential temperature response index; IL, interleukin; IM, intramuscular; LPS, lipopolysaccharide; TRI, thermal response index; TNZ, thermoneutral zone

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phase (mice) (Rudaya et al., 2005). Several bird species including pigeons (Nomoto, 1996, 2003), chickens (DeBoever et al., 2008; Fraifeld et al., 1995; Johnson et al., 1993b; Jones et al., 1983), ducks (Maloney and Gray, 1998), and free-living song sparrows (Adelman et al., 2010) develop fevers in response to an injection of LPS when kept in thermoneutral/natural environments. Burness et al. (2010) showed that Zebra finches do not mount fevers after being given lipopolysaccharide, when they are kept in cool (15 °C) or in hot (34 °C) environments (Burness et al., 2010). Whether the lack of a response outside the thermoneutral zone (TNZ) is a characteristic of Zebra finches, or more generally applicable, is not known.

We investigated the influence of ambient temperature on the febrile response in female Pekin ducks. Pekin ducks are phylogenetically old birds from the order galloanserine, which is thought to be the basal lineage from which modern birds developed (vanTuinen et al., 2000). It is therefore plausible that the physiological mechanisms responsible for fever in Pekin ducks extend to other bird species. In fact, like ducks, chickens and pigeons employ prostaglandins for febrile mediation and glucocorticoids for febrile modulation (Gray et al., 2005; Johnson et al., 1993b; Klasing, 1998; Macari et al., 1993; Nomoto, 2003).

The aim of our study was to investigate the characteristics of the febrile response in female Pekin ducks following injection of lipopolysaccharide (LPS) at ambient temperatures that impose different demands on the thermoregulatory system. We found that in female Pekin ducks, low temperatures inhibit, whereas high ambient temperatures augment, the febrile response to LPS. This may have important implications for the survival of avian species in changing climates.

2. Materials and Methods

2.1. Ethical approval

The procedures of this study were approved by the Animal Ethics Committee of the University of the Witwatersrand (application no.: 2008/31/04 and 2010/05/05).

2.2. Experimental design

The research consisted of two study components: for the first study we investigated the influence of ambient temperature on the febrile response in female Pekin ducks. Once we found that ambient temperature affected the magnitude and duration of the febrile responses, we initiated a second study and exposed ducks to conditions that could prevail during one day of a heat wave (Boeck et al., 2010; D'Ippoliti et al., 2010; Kyselý and Dubrovsky, 2005).

Each study was completed over a period of three months. For the first study we followed the protocol of Stitt (1973) for our experimental design. Stitt (1973) investigated the effect of ambient temperature on the febrile response in rabbits. Consequently, the results of that study provided a comparative model for our investigations.

For the second study we simulated a heat wave using the records that describe air temperature and humidity changes on a day when a heat wave prevailed in the temperate zone of the northern hemisphere, which is considered these ducks' natural environment (Boeck et al., 2010; D'Ippoliti et al., 2010; Kyselý, 2002; Kyselý and Dubrovsky, 2005).

2.3. Animals

We used 10 female Pekin ducks (*Anas platyrhynchos*) for the first study and 6 female Pekin ducks for the second study. The group of ducks assigned to the first study had a mean body mass of 2.8 ± 0.2 kg and the ducks assigned to the second study had a mean body mass of 2.9 ± 0.3 kg The ducks were housed as a flock in an indoor room and exposed to a 12:12 h light:dark cycle with lights on at 06:00 and an ambient temperature of 20 ± 1 °C—a temperature that falls within the TNZ of Pekin ducks (Bouverot et al., 1974; Nichelmann, 1983; Nichelmann and Tzschentke, 2002; Simon-Opperman et al., 1978). The ducks had *ad libitum* access to water and dry chicken food enriched with minerals and vitamins.

2.4. Body temperature measurements

Core body temperature was measured with abdominally implanted miniature temperature data loggers (Tidbit, Onset Computer, Bourne, MA, USA). The loggers have a resolution of 0.02 °C (Tidbit, Onset Computer, Bourne, MA, USA) and were calibrated (from 38 to 44 °C at 1 °C intervals) against a certified precision thermometer (Quat 100, Heraeus, Germany). They were coated with wax (Sasol Wax, Sasolburg, RSA) before implantation. For implantation the ducks were anaesthetised with a gaseous induction of 2% Isoflurane (Safe Line Pharmaceuticals, Wadeville, JHB, RSA) in oxygen for data logger implantation. We commenced experimental procedures three weeks after data logger implantation.

2.5. Manipulation of ambient temperature

2.5.1. Study 1, the influence of ambient temperature on fever

Ducks were involved in experiments that required manipulation of ambient temperature once every two weeks. On the days of experimentation the ducks were placed in a temperature control room for 12 h (from 09:00 to 21:00). This room looked similar to the pen in which the ducks usually lived, and the ducks had *ad libitum* access to dry chicken feed, and drinking as well as bathing water. The temperature of the room was pre-set to one of the three temperatures: (1) 25 °C, a temperature that was reported by Simon-Opperman et al. (1978) as within the TNZ of Pekin ducks, (2) 33 °C and (3) 6 °C—temperatures that are higher and lower than the TNZ of these birds (Bouverot et al., 1974). Care was taken to ensure that the room's temperature stayed constant throughout the experiment.

2.5.2. Study 2, fever during a simulated heat wave

Individual ducks were randomly chosen from the flock and placed in a temperature control chamber (SB300, Weiss Umwelttechnik, Berlin, Germany) for 24 h (from 18:00 until 18:00 the following day). The ducks had *ad libitum* access to dry chicken feed, and drinking as well as bathing water. The chamber was big enough (1.7 m $\times$ 0.8 m $\times$ 1.6 m) for the ducks to comfortably sit or stand and reposition. An air compressor (B100, Charles Austen Pumps Ltd., Surrey, UK) placed outside the chamber supplied 20 L/min of fresh air to the chamber. Air flowing into the chamber was directed through a 2 m long copper coil placed on the inside of the chamber, so that the temperature of air flowing into the chamber was always the same as the temperature of the chamber. From 18:00 until 08:00 the temperature of the chamber was 25 °C, thereafter the temperature of the chamber was increased and reached 40 °C by 12:00 (see Fig. 2). We kept the temperature at 40 °C until 14:00 and then lowered it to reach 25 °C by 16:00. The relative humidity of the air inside the chamber was monitored to not exceed 50%.

2.6. Injections

2.6.1. Study 1, the influence of ambient temperature on fever

On the days of experimentation the ducks were given an intramuscular injection of either pyrogen or saline at 11:00. To evoke fevers, 100 μ kg^{-1} LPS from *Escherichia coli* (Sigma-Aldrich,