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Melatonin treatment counteracts the hyperthermic effect of lipopolysaccharide injection in the Syrian hamster

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Abstract

The present study examined the acute response in body temperature to lipopolysaccharide (LPS) injection to Syrian hamsters at two time intervals during the light-dark cycle. Its modification by melatonin (MT) administration in the drinking water was also assessed. Hamsters were intraperitoneally (i.p.) implanted with a transmitter to measure core body temperature. MT was administered from day 8 post-surgery until the end of experiment. On day 16 after surgery, LPS or saline was injected i.p. at the beginning of the light phase (ZT 0) or of the scotophase (ZT 14). At ZT 0, LPS increased core body temperature, an effect that persisted for at least 5 h and that was blunted by MT administration. At ZT 14, the hyperthermic effect of LPS was absent. Rather, at ZT 14 the animals showed increases in core body temperature following saline or LPS during the first 2 h after injection only, which were significantly less intense in LPS-treated animals. MT administration blunted this difference. Five days after injection, hamsters that had received LPS at ZT 0 showed an increase in the mesor of core body temperature rhythm as compared to saline. This effect was suppressed by MT administration. The results demonstrate that MT prevents body temperature increase after LPS at ZT 0.

Keywords: Lipopolysaccharide; Melatonin; Hyperthermia; Septic shock; Syrian hamster

Lipopolysaccharide (LPS) is a well-conserved cell wall component of Gram-negative bacteria known for its ability to induce a septic shock as a result of an overwhelming secretion of cytokines. After binding to immunocytes, LPS initiates a signalling cascade that leads to transcriptional activation of nuclear factor κB and finally to the up-regulation, among other gene products, of the inflammatory cytokines interleukins 1 and 6 and tumor necrosis factor-α [15,21].

Inflammatory cytokines act both directly and indirectly on the brain (by an effect on the circumventricular organs and via the vagus nerve, respectively) to trigger "sickness behavior" [1,2,5,8,10]. This syndrome includes the increase of body temperature, sleep propensity, a modified brain neurotransmitter dynamics, activation of the hypothalamic-pituitary-adrenocortical axis and decreases of locomotor activity, feeding, drinking and social interactions [6,9]. Sickness behavior is inducible by the systemic administration of LPS [1].

Body temperature, a physiological variable that can be telemetrically monitored under laboratory conditions, is one of the physiological functions that shows a daily variation amenable of modification during sickness behavior [14,17–19]. Among the endogenous signals known to affect circadian rhythmicity, the pineal product melatonin (MT) plays a role as a modulator of the neuroimmuno-endocrine system [11,13]. MT is also an effective free radical scavenger and antioxidant at physiological and pharmacological concentrations [16]. Both properties of MT may be involved in its protective effect on LPS-induced sickness behavior [20].

The objective of the present study was to assess the effect of MT, administered in the drinking water, on acute responses in body temperature after LPS injection to Syrian hamsters either at the beginning of the light phase (ZT 0) or at the begin-

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ning of the scotophase (ZT 14). The effect of treatment on the circadian rhythm of body temperature was also assessed 5 days after the injection.

Adult male Syrian hamsters (LVG, Charles Rivers, USA) raised in our colony (body weight ≅ 150 g) were housed individually and were maintained on a 14:10 light-dark cycle (lights off at 20:00 h) in a sound-attenuated and temperature controlled (20–22 °C) room. Food and water were given "ad libitum". To record body temperature, animals were intraperitoneally (i.p.) implanted with a transmitter (Dataquest—Mini-Mitter, MN, USA). Animals were anesthetized with equitesine (0.4 ml/100 g BW). One week was allowed to recovery from surgery. During the first 3 days post-surgery, animals received antibiotic and analgesic treatment.

Efforts were made to minimize pain or discomfort, in accordance with the principles and procedures outlined in European Communities Council Directives (86/609/EEC). Body temperature was recorded continuously in 10 min bins during all experiment. Data were analyzed using the Circadia program (Department of Psychology, Simon Fraser University, Canada). MT was administered in drinking water starting on day 8 post-surgery until the end of experiment. MT (kindly provided by Elisium S.A., Buenos Aires, Argentina) was dissolved in absolute ethanol and diluted in sterile water for a final concentration of 25 μg/ml. Previous works from our laboratory, using the same MT dose, resulted in a 50-fold-increase in plasma melatonin levels [3]. Control animals were given solvent (1% ethanol in water).

On day 16 after surgery, LPS (30 μg/kg, Sigma Chemical Co., St. Louis, MO, USA) or saline was administered i.p. This dose was chosen after assessing that it was enough to elevate body temperature without producing shock. The injection was done either 5 min before the beginning of the dark phase (ZT 14) or 5 min before the onset of the light phase (ZT 0). Animals were divided into eight experimental groups ($n = 8$ per group) as follows: MT–LPS-ZT 14; MT–LPS-ZT 0; MT–saline-ZT 14; MT–saline-ZT 0; vehicle–LPS-ZT 14; vehicle–LPS-ZT 0; vehicle–saline-ZT 14; vehicle–saline-ZT 0.

Statistical analysis of results was made by using a Repeated Measures ANOVA. Treatment (LPS versus saline), melatonin (MT or vehicle) and time of administration (ZT 0 or ZT 14) were defined as inter-subject factors, whereas period (pre-treatment versus post-treatment) was defined as an intra-subject factor. Main effects as well as interactions were analyzed. Meaningful post hoc comparisons were performed by Repeated Measures ANOVA with treatment as inter-subject factor and period as intra-subject factor. Data are shown as mean ± S.E.M. The Pearson method was employed to calculate correlation coefficients between body temperature and locomotor activity.

Fig. 1 depicts the effect of MT on changes in core body temperature caused by LPS within 2 h after injection at ZT 0 or ZT 14. Repeated Measures ANOVA indicated a significant interaction among LPS, MT and time of injection ($F = 8.6$, $p = 0.005$). At ZT 14, hamsters that received saline or LPS showed increases in core body temperature which were significantly less intense in LPS-treated animals ($F = 4.9$, $p = 0.045$, Fig. 1). MT treatment blunted this difference ($F = 0.02$, $p = 0.9$). Hamsters injected with LPS at ZT 0 showed increased core body temperature for the next 2 h ($F = 15.0$, $p = 0.002$), an effect again blunted by MT administration ($F = 1.0$, $p = 0.6$, Fig. 1).

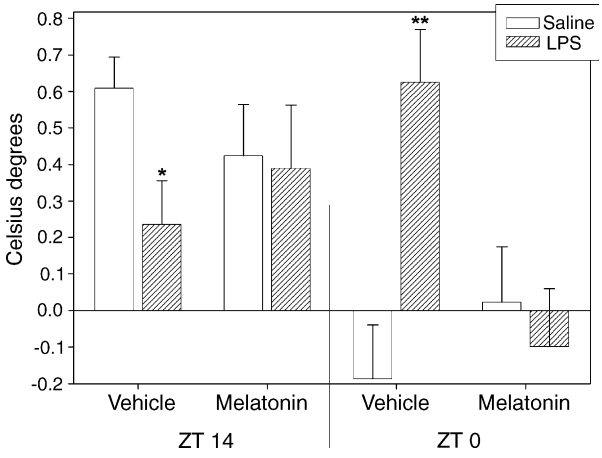

Fig. 1. Changes in body temperature during the first 2 h after i.p. injection of LPS or saline to hamsters that were receiving or not MT in the drinking water. Comparisons were made with the same 2 h period of the precedent day. Injections were done at the light-dark (ZT 14) or dark-light (ZT 0) transitions. Data are expressed as differences in Celsius degrees between post- and pre-treatment. Values are means ± S.E.M. ($n = 8$/group). $^{*}p = 0.05$; $^{**}p = 0.001$ vs. vehicle.

Fig. 2 shows the effect of MT on LPS-induced changes in body temperature 5 h after injection. Repeated Measures ANOVA revealed a significant interaction among LPS, MT and time of injection ($F = 7.7$, $p = 0.008$). The hyperthermic effect of LPS was significant at ZT 0 only ($F = 20.0$,

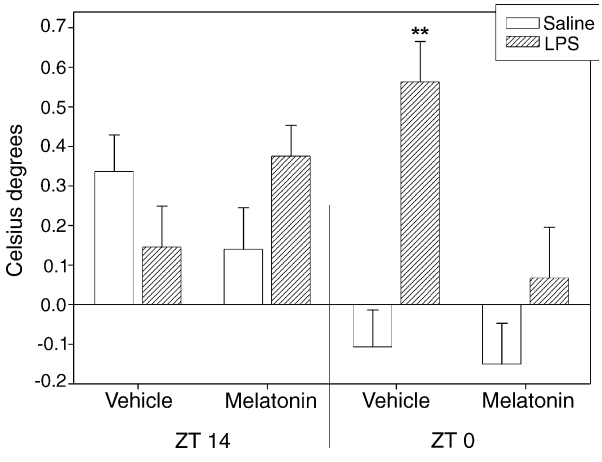

Fig. 2. Differences in body temperature during the first 5 h post-injection after i.p. LPS or saline administration to animals that received or not MT in the drinking water. Comparisons were made with the same 5 h period of the precedent day. Injections were done at the light-dark (ZT 14) or dark-light (ZT 0) transitions. Data are expressed as differences in Celsius degrees between post- and pre-treatment. Values are means ± S.E.M. $^{**}p = 0.001$ vs. vehicle.