Journal of Thermal Biology 85 (2019) 102411

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Splenic anti-inflammatory reflex in immune tolerance

Jonatas E. Nogueira[a], Brenda K.L. Agostinho[b], Clarissa M.D. Mota[c], Luiz G.S. Branco[b,c,*]

[a] School of Physical Education and Sports of Ribeirao Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
[b] Department of Basic and Oral Biology, Dental School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
[c] Department of Physiology, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Keywords:
Endotoxin
LPS
Fever
Cytokines
PGE_2

ABSTRACT

The injection of repeated doses of lipopolysaccharide (LPS) results in attenuation of the immune response, which is an important mechanism to prevent deleterious long-term excessive inflammation. Brain-mediated mechanisms are involved in this endogenous anti-inflammatory effect, but nothing is known about the putative role of the splenic anti-inflammatory reflex (which has recently been described as a powerful mechanism involved in the suppression of immune response) during immune tolerance. Therefore, we tested the hypothesis that endotoxin tolerance is at least in part mediated by the splenic anti-inflammatory reflex. Body core temperature (Tb) was measured in rats previously submitted to splenectomy. Immune tolerance was induced by means of five consecutive LPS (100 μg/kg) intraperitoneal injections at 24-h intervals. In sham operated rats, we observed a significant reduction of the febrile response to repeated administration of LPS, which was not altered in rats submitted to splenectomy. Moreover, plasma pro-inflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6] and prostaglanding E_2 (PGE_2) surges besides preoptic PGE_2 levels were observed after the first LPS administration but not in tolerant animals, and this pattern was kept the same in splenectomized rats. These data are consistent with the notion that the splenic anti-inflammatory reflex does not modulate immune tolerance in rats.

1. Introduction

Circulating endotoxins, such as lipopolysaccharides (LPS), cause fever, besides behavioral changes that are commonly referred to as sickness syndrome. This condition is triggered by a number of microorganisms that release endotoxins and activate immune cells to produce inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 and eicosanoids such as prostaglandins, including prostaglandin E_2 (PGE_2) (Roth and Blatteis, 2014). These febrigenic mediators acting directly or indirectly eventually increase preoptic area PGE_2 levels, which is the proximal mediator of fever (Feleder et al., 2007). Such microorganisms may act at different body tissues they infect but the outcoming response is typically constituted by a unique set of symptoms that may be unpleasant but are often effective restoring health (Saper et al., 2012).

Interestingly, pre exposure to low doses of LPS attenuates sickness syndrome after a subsequent challenge with LPS, which is controlled by a number of different mechanisms (Dias et al., 2005; Piotrowski et al., 2014). The so called endotoxin tolerance also occur in humans, being an important mechanism protecting the host against excessive response to prolonged exposure to endotoxin (Draisma et al., 2009; Kox et al., 2011; Perry et al., 2005).

The inflammatory response must be precisely regulated since an uncontrolled inflammatory response results in clinical complications such as septic shock, autoimmune diseases and cancer. Locally, the inflammatory response is essentially regulated by pro- and anti-inflammatory cytokines production by immune cells, whereas systemically this response is controlled mainly by a sophisticated mechanism involving neuro-immune interaction (Chavan et al., 2017). It has been documented an important relation between vagus nerve and the innate immune system, which has been referred as the "vagal cholinergic anti-inflammatory reflex", *i.e.*, an effective link between the nervous and the immune systems that is essential to regulate inflammation (Martelli et al., 2014; Okusa et al., 2017; Tracey, 2002). The cholinergic anti-inflammatory pathway has been reported to be mediated by the spleen, through modulation of specific nicotinic receptors (α7 subunit of naChr) which are expressed in immune cells (Borovikova et al., 2000). In agreement with this notion, α7naChr selective agonists (Huang et al., 2008), similarly to vagus nerve stimulation (Tracey, 2002), leads to reduced production of pro-inflammatory cytokines during systemic inflammation.

Since there is no information about the putative role of the splenic

* Corresponding author. Department of Basic and Oral biology, Dental School of Ribeirão Preto, University of São Paulo, 14040-904, Ribeirão Preto, SP, Brazil.
E-mail address: branco@forp.usp.br (L.G.S. Branco).

https://doi.org/10.1016/j.jtherbio.2019.102411
Received 14 June 2019; Received in revised form 29 August 2019; Accepted 3 September 2019
Available online 04 September 2019
0306-4565/

anti-inflammatory reflex on the development of immune tolerance, in the present study, we tested the hypothesis that the splenic anti-inflammatory reflex participates of the immune tolerance, analyzing not only the febrile response but also the levels of the plasma cytokines TNF-α, IL-1β and IL-6 and PGE_2, and preoptic (the hierarchically most important region for thermoregulation) PGE_2 levels (as the proximal mediator of fever) in rats.

2. Methods

2.1. Animals

All experimental procedures were performed in accordance to the Guide for the Care and Use of Laboratory Animals of the National Council for the Control of Animal Experimentation (CONCEA) and were approved by the Local Animal Ethical Committee of the Dental School of Ribeirao Preto, University of Sao Paulo (Protocol number 2016.1.393.58.1). Adult male Wistar Hannover rats (250–270 g, 7–8 weeks) were individually caged and maintained at a controlled temperature of 23 ± 1 °C on a 12 h light/dark cycle (lights on at 6 h am), with free access to food and water (*ad libitum*).

2.2. Surgeries

Five days before the experiments, the rats were anesthetized with ketamine-xylazine (100 and 10 mg/kg, respectively; 1 ml/kg, intraperitoneal, i.p.) and submitted to a splenectomy based on previous study (Huston et al., 2006). An abdominal incision in the epigastrium and mesogastrium was performed and the spleen was exposed. Then, the three main branches of the spleen artery were stabilized with nylon thread, ligated, cut and the spleen was removed. After that, a temperature datalogger capsule (SubCue, Calgary, Alberta, Canada) was placed into the peritoneal cavity and the wound was sutured. After the surgical procedure, a dose of antibiotic (Pentabiotc; 24,000 UI/kg, Zoetis, Brazil, intramuscularly) and analgesic (Niglumine; 2.5 mg/kg, subcutaneously) medication was provided.

2.3. Deep body temperature (Tb) measurements

Tb of the rats was measured by the datalogger at 5-min intervals, during a period of 1 h before and 5 h after the treatments with LPS or saline.

2.4. Drugs

LPS derived from *Escherichia coli* (serotype 0111: b4) was dissolved in sterile 0.9% sodium chloride (saline).

2.5. Experimental protocols

The experiments were conducted 5 days after the splenectomy. In all experimental protocols the rats remained individually in a room at an ambient temperature (Ta) considered as the thermoneutral zone (29 °C) (Steiner and Branco, 2002). Splenectomized rats received LPS (100 μg/kg, i.p.) dissolved in saline. Saline was injected into another group of splenectomized rats as control (1 ml/kg, i.p.). Non-splenectomized rats (sham groups) received the same treatment, *i.e.*, injection of either LPS or saline. The LPS dose was chosen based on previous studies (Almeida et al., 1999; Raffaini et al., 2006; Soriano and Branco, 2010).

2.5.1. Protocol 1

This experimental protocol was designed to assess plasma cytokines (TNF-α, IL-1β and IL-6) and PGE_2 levels, and PGE_2 levels in the preoptic area of the hypothalamus at 5 h after LPS or saline administration. LPS (100 μg/kg, i.p.) or saline (1 ml/kg, i.p.) was injected in splenectomized or sham operated rats. Tb was measured during a period of 1 h before and 5 h after the treatment. After that, the animals were euthanized in order to collect blood samples and the brains.

2.5.2. Protocol 2

The second experimental protocol aimed to evaluate endotoxin tolerance after LPS injection for five consecutive days. LPS (100 μg/kg, i.p.) or saline (1 ml/kg, i.p.) was injected in splenectomized or sham rats. Tb was measured during a period of 1 h before and 5 h after the treatments for five days. Then, the rats were euthanized and the blood was collected and processed in order to assess plasma cytokines (TNF-α, IL-1β and IL-6) and PGE_2 levels, as described below. In addition, the brains were collected to measure PGE_2 levels in the preoptic area of the hypothalamus.

2.6. Plasma and preoptic area of the hypothalamus samples collection

Blood samples were collected in heparin-coated tubes. Then, the tubes were centrifuged at 3.500 rpm for 20 min at 4 °C and plasma was stored at −70 °C.

Brains were quickly excised, promptly frozen by submersion in dry ice-cold isopentane, and stored at −70 °C. Preoptic area of the hypothalamus were sampled in a cryostat by a punch needle (0.9 mm inner diameter) from a 500-μm thick slice based on the following landmarks: ventral, optic chiasm; dorsal, anterior commissure; median, the 3 V (Paxinos and Watson, 2005).

2.7. Cytokines and PGE_2 assays

Plasma TNF-α, IL-1β, and IL-6 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) kits for each cytokine (R& D Systems, Minneapolis, Minn., USA) according to the manufacturer's instructions. Plasma PGE_2 levels were determined using ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

Preoptic PGE_2 samples were homogenized in phosphate-buffered saline (PBS, 0.01M at pH 7.4), containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 10 μg/mL indomethacin and centrifuged at 13,000 rpm for 10 min at 4 °C and the supernatants collected to analysis. Preoptic PGE_2 levels were determined using ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Protein concentrations in the supernatants were measured using the Bradford method (Bradford, 1976) and used to normalize the results. These procedures are based in previous studies (Mota et al., 2017; Saramago et al., 2019).

2.8. Statistical analyzes

All data in the figures are expressed as mean ± standard error of the mean (SEM). Tb values (°C) plotted at 5-min intervals are shown as raw values. Thermal index (TI), expressed as °C × min, were calculated from area under curve (AUC) of values of Tb during the febrile phase (120–300 min). Baseline was set at 36.5 °C. Statistical differences among groups were determined by one-way ANOVA followed by Bonferroni post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. LPS-induced endotoxin tolerance in rats

We investigated the development of endotoxin tolerance in splenectomized and sham operated rats after five consecutive LPS administration (Fig. 1). Intraperitoneal injection of saline caused no changes in Tb in any experimental group (Fig. 1A–E). On the other hand, i.p. administration of LPS caused the typical febrile response in splenectomized and sham operated rats on the first day (Fig. 1A). On the second

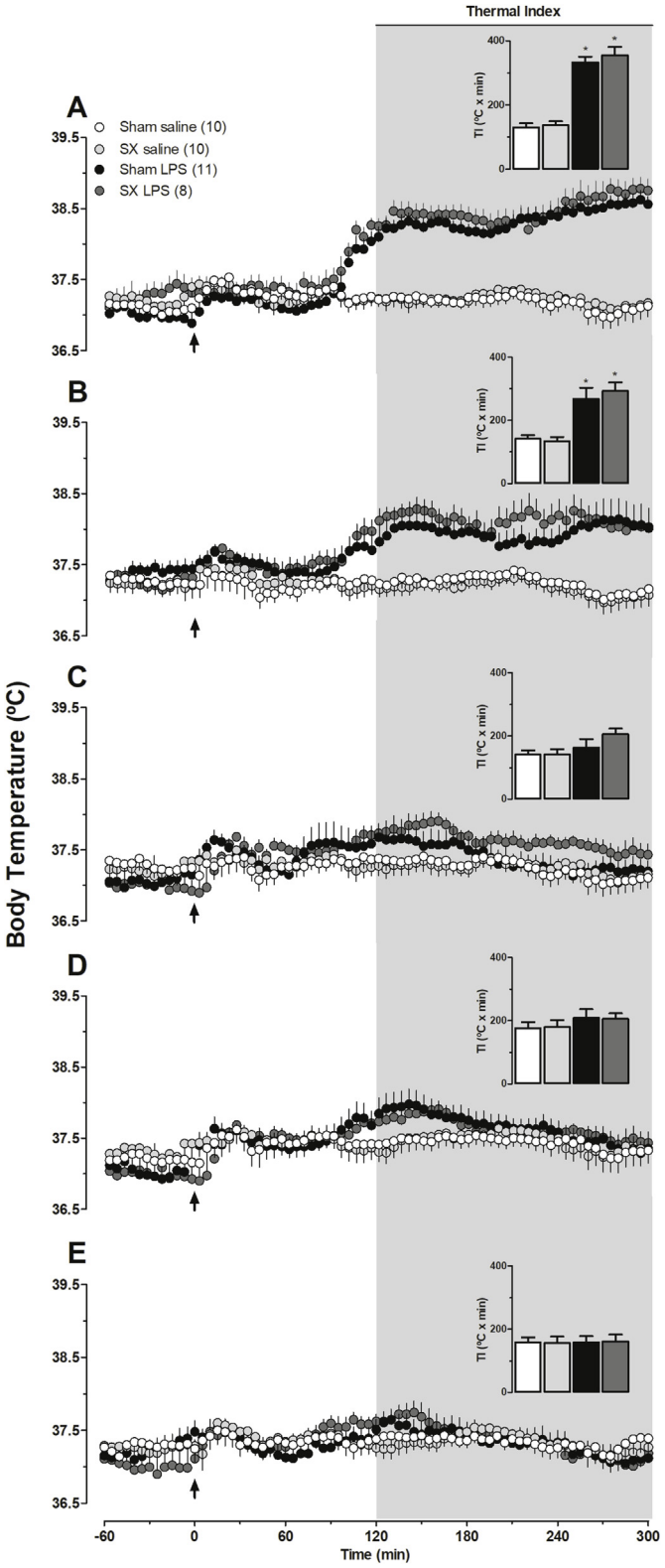

Fig. 1. Time courses of body temperature (Tb) of Sham saline, splenectomy (SX) saline, Sham LPS, and SX LPS groups on day 1 (A), 2 (B), 3 (C), 4 (D) and 5 (E). Arrow indicate the time of injection of saline or LPS intraperitoneally daily. The gray area indicates the febrile period analyzed by thermal indexes (120–300 min). Thermal indexes of the febrile periods between saline and LPS-treated groups are represented on the upper right corner of the time-course graph in each day. Data are presented as mean ± SEM. *p < 0.05, groups treated with LPS vs. groups treated with saline.

day of LPS administration, the animals presented a change in the febrile response (Fig. 1B), indicating the start of the development of endotoxin tolerance. On the third, fourth and fifth days of LPS injection, fever response was significantly blunted and endotoxin tolerance was completed (Fig. 1C–E). Thermal indexes (TI) of febrile phase (indicated by the gray area, Fig. 1A–E) were calculated to clarify the changes in Tb observed in splenectomized and sham operated rats. As shown in Fig. 1A and B, LPS caused a significant ($p < 0.05$) increase in TI (fever) of splenectomized and sham rats while the groups treated with saline had no significant changes in TI. Fig. 1C–E shows that both LPS and saline administration did not change TI in any experimental groups.

3.2. Cytokines and PGE_2 production in immune tolerant rats

To investigate the effects of the development of endotoxin tolerance on plasma febrigenic signaling, we measured plasma levels of inflammatory cytokines (TNF-α, IL-1β and IL-6) and PGE_2 in splenectomized and sham operated rats injected with LPS or saline. After the first LPS injection, we observed typical surges of plasma inflammatory cytokines and PGE_2 that were not affected by splenectomy (Fig. 2, left panels). On the other hand, the inflammatory cytokines (TNF-α, IL-1β and IL-6) and PGE_2 levels could not be detected on the fifth day of LPS treatment or saline injections (Fig. 2, right panels). Similar results were observed when analyzing the preoptic PGE_2 levels that were increased after the first LPS administration but not in any other experimental group (Fig. 3), each is associated with the observed changes in Tb (Fig. 1).

4. Discussion

The present study is the first to report that the splenic anti-inflammatory reflex plays no role in immune tolerance, based on the fact splenectomy failed to cause any change in the absent febrigenic signaling and fever in tolerant rats (Figs. 1–3).

Paul Beeson in 1946 (Beeson, 1946) first documented that rabbits previously exposed to a sublethal dose of bacterial endotoxin subsequently survive a lethal dose of endotoxin. We now know that a myriad of mechanisms are involved in this refractory state; however, a complete understanding of this phenomenon remains elusive. On the other hand, it is now clear that endotoxin tolerance is associated with the innate immune system and particularly with macrophages (Cavaillon and Adib-Conquy, 2006), that are a cell type intrinsically implicated with the splenic anti-inflammatory reflex.

During systemic inflammation, the production of pro-inflammatory mediators is down-modulated by the “inflammatory reflex”, which requires the activation of neural circuits that inhibit cytokine production in peripheral organs (Martelli et al., 2014; Okusa et al., 2017; Tracey, 2002), that is the basis of the splenic anti-inflammatory reflex. It is now well accepted that this reflex rely on efferent neural connections from the brain to the viscera, including the spleen, reducing the production of pro-inflammatory cytokines (Okusa et al., 2017). As any other reflex, the “inflammatory reflex” relies on afferent pathways, and in this case vagal afferent visceral terminals that are activated by circulating cytokines (Komegae et al., 2018). The vagal afferent fibers projects to the nucleus of the solitary tract (NTS) (Berthoud and Neuhuber, 2000) before affecting other brain regions (Chavan et al., 2017), that act together increasing sympathetic tone (Tkacs and Strack, 1995) which increases the release of noradrenaline in the preoptic region of the hypothalamus (the hierarchically most important region for Tb control) during LPS-induced immune challenge (Villanueva et al., 2009). NTS noradrenergic projections to the preoptic area of the hypothalamus (Palkovits et al., 1980) are known to play a key role in the modulation of fever (Feleder et al., 2007). This higher sympathetic tone also affects the spleen since an increased activity of the splenic nerves is observed (MacNeil et al., 1997) which is part of the splenic anti-inflammatory reflex (Okusa et al., 2017; Tracey, 2002). The present study does

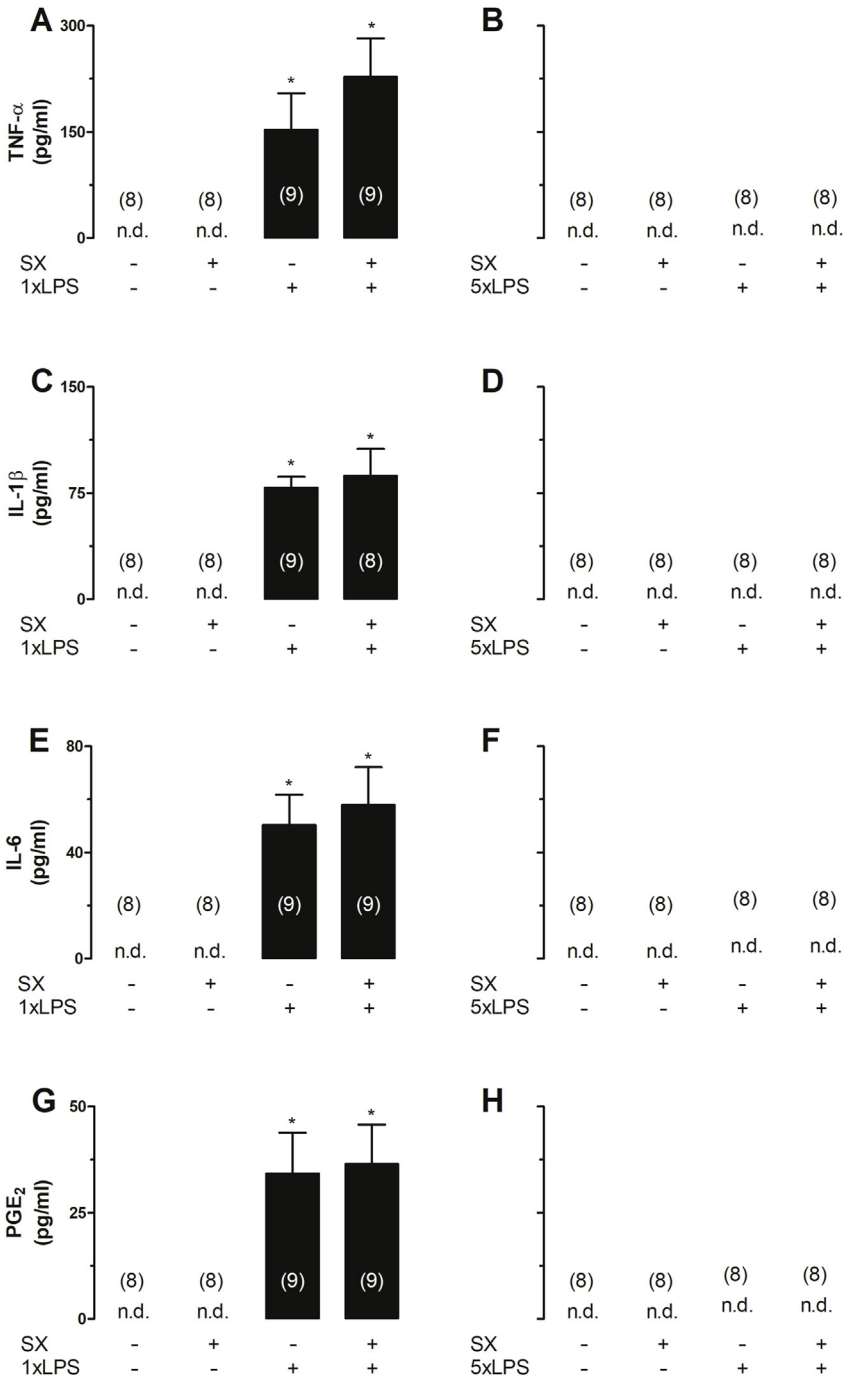

Fig. 2. Plasma levels of TNF-α (A, B); IL-1β (C, D); IL-6 (E, F) and PGE_2 (G, H). Samples from Splenectomy (SX) or Sham groups treated with saline (1 ml/kg, ip) or LPS (100 μg/kg, ip) once or for five days. Samples were obtained 300 mim after administration. Data are presented as mean ± SEM. n.d., Nondetectable levels. *p < 0.05, groups treated with LPS vs. groups treated with saline.

corroborate previous data specifically about the observed LPS-induced TNF-α surges in splenectomized rats (Vida et al., 2011) that are observed to be unaltered in comparison to sham operated rats. These deceptive negative results may be related to the different doses of LPS and the time for blood sampling after LPS administration. More importantly, this study adds new evidences that this important reflex is silent during immune tolerance (Figs. 1 and 2, right panels).

5. Conclusion

Immune tolerance takes place by a complex set of mechanisms (Draisma et al., 2009) that are orchestrated specifically to cause a drastic decrease in the production of the inflammatory mediators. Several recent reports show that clinical examples of endotoxin tolerance comprise not only sepsis but also other diseases like cystic fibrosis (del Fresno et al., 2008), acute coronary syndrome (del Fresno et al., 2007), and trauma (Lin et al., 2007) that are conditions in which the risk of new infections correlates with a refractory state. This study sheds light on this scenario providing information about the absence of a role for the splenic anti-inflammatory reflex during entodoxin tolerance.

Conflicts of interest

We have no conflict of interest to declare.

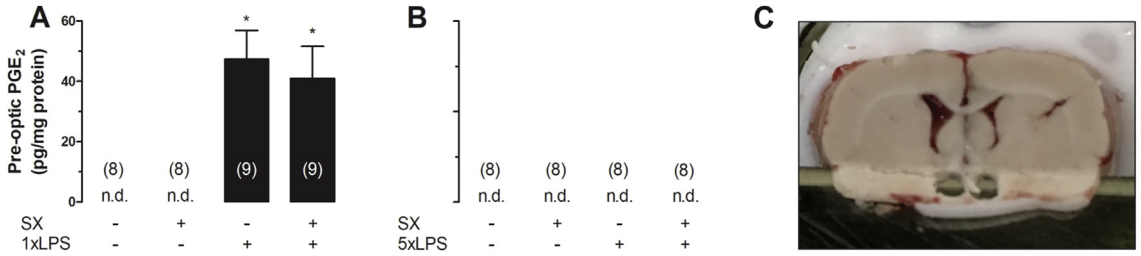

Fig. 3. Preoptic PGE_2 levels of Splenectomy (SX) or Sham groups treated with saline (1 ml/kg, ip) or LPS (100 μg/kg, ip) once (A) or for five days (B). Representative photo of a punch in the preoptic area (C). Samples were obtained 300 mim after administration. Data are presented as mean ± SEM. n.d., Nondetectable levels. *p < 0.05, groups treated with LPS vs. groups treated with saline.

Financial support

This work was supported by Grants #16/17681-9 São Paulo Research Foundation (FAPESP), and National Council for Scientific and Technological Development (CNPq); Brazil. C.M.D.M. receives a fellowship by FAPESP (#17/01633-8).

Acknowledgments

The authors acknowledge Mrs. Nadir Fernandes and Mr. Mauro F. Silva for technical support.

References

Almeida, M.C., Trevisan, F.N., Barros, R.C., Carnio, E.C., Branco, L.G., 1999. Tolerance to lipopolysaccharide is related to the nitric oxide pathway. Neuroreport 10, 3061–3065.
Beeson, P.B., 1946. Development of tolerance to typhoid bacterial pyrogen and its abolition by reticulo-endothelial blockade. Proc. Soc. Exp. Biol. Med. 61, 248–250 Society for Experimental Biology and Medicine.
Berthoud, H.R., Neuhuber, W.L., 2000. Functional and chemical anatomy of the afferent vagal system. Auton. Neurosci. : basic & clinical 85, 1–17.
Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 405, 458–462.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Cavaillon, J.M., Adib-Conquy, M., 2006. Bench-to-bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis. Crit. Care 10, 233.
Chavan, S.S., Pavlov, V.A., Tracey, K.J., 2017. Mechanisms and therapeutic relevance of neuro-immune communication. Immunity 46, 927–942.
del Fresno, C., Gomez-Pina, V., Lores, V., Soares-Schanoski, A., Fernandez-Ruiz, I., Rojo, B., Alvarez-Sala, R., Caballero-Garrido, E., Garcia, F., Veliz, T., Arnalich, F., Fuentes-Prior, P., Garcia-Rio, F., Lopez-Collazo, E., 2008. Monocytes from cystic fibrosis patients are locked in an LPS tolerance state: down-regulation of TREM-1 as putative underlying mechanism. PLoS One 3, e2667.
del Fresno, C., Soler-Rangel, L., Soares-Schanoski, A., Gomez-Pina, V., Gonzalez-Leon, M.C., Gomez-Garcia, L., Mendoza-Barbera, E., Rodriguez-Rojas, A., Garcia, F., Fuentes-Prior, P., Arnalich, F., Lopez-Collazo, E., 2007. Inflammatory responses associated with acute coronary syndrome up-regulate IRAK-M and induce endotoxin tolerance in circulating monocytes. J. Endotoxin Res. 13, 39–52.
Dias, M.B., Almeida, M.C., Carnio, E.C., Branco, L.G., 2005. Role of nitric oxide in tolerance to lipopolysaccharide in mice. J. Appl. Physiol. 98, 1322–1327 1985.
Draisma, A., Pickkers, P., Bouw, M.P., van der Hoeven, J.G., 2009. Development of endotoxin tolerance in humans in vivo. Crit. Care Med. 37, 1261–1267.
Feleder, C., Perlik, V., Blatteis, C.M., 2007. Preoptic nitric oxide attenuates endotoxic fever in Guinea pigs by inhibiting the POA release of norepinephrine. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R1144–R1151.
Huang, X., Zheng, F., Stokes, C., Papke, R.L., Zhan, C.G., 2008. Modeling binding modes of alpha7 nicotinic acetylcholine receptor with ligands: the roles of Gln117 and other residues of the receptor in agonist binding. J. Med. Chem. 51, 6293–6302.
Huston, J.M., Ochani, M., Rosas-Ballina, M., Liao, H., Ochani, K., Pavlov, V.A., Gallowitsch-Puerta, M., Ashok, M., Czura, C.J., Foxwell, B., Tracey, K.J., Ulloa, L., 2006. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J. Exp. Med. 203, 1623–1628.
Komegae, E.N., Farmer, D.G.S., Brooks, V.L., McKinley, M.J., McAllen, R.M., Martelli, D., 2018. Vagal afferent activation suppresses systemic inflammation via the splanchnic anti-inflammatory pathway. Brain Behav. Immun. 73, 441–449.
Kox, M., de Kleijn, S., Pompe, J.C., Ramakers, B.P., Netea, M.G., van der Hoeven, J.G., Hoedemaekers, C.W., Pickkers, P., 2011. Differential ex vivo and in vivo endotoxin tolerance kinetics following human endotoxemia. Crit. Care Med. 39, 1866–1870.
Lin, C.Y., Tsai, I.F., Ho, Y.P., Huang, C.T., Lin, Y.C., Lin, C.J., Tseng, S.C., Lin, W.P., Chen, W.T., Sheen, I.S., 2007. Endotoxemia contributes to the immune paralysis in patients with cirrhosis. J. Hepatol. 46, 816–826.
MacNeil, B.J., Jansen, A.H., Janz, L.J., Greenberg, A.H., Nance, D.M., 1997. Peripheral endotoxin increases splenic sympathetic nerve activity via central prostaglandin synthesis. Am. J. Physiol. 273, R609–R614.
Martelli, D., Yao, S.T., McKinley, M.J., McAllen, R.M., 2014. Reflex control of inflammation by sympathetic nerves, not the vagus. J. Physiol. 592, 1677–1686.
Mota, C.M.D., Rodrigues-Santos, C., Fernandez, R.A.R., Carolino, R.O.G., Antunes-Rodrigues, J., Anselmo-Franci, J.A., Branco, L.G.S., 2017. Central serotonin attenuates LPS-induced systemic inflammation. Brain Behav. Immun. 66, 372–381.
Okusa, M.D., Rosin, D.L., Tracey, K.J., 2017. Targeting neural reflex circuits in immunity to treat kidney disease. Nat. Rev. Nephrol. 13, 669–680.
Palkovits, M., Zaborszky, L., Feminger, A., Mezey, E., Fekete, M.I., Herman, J.P., Kanyicska, B., Szabo, D., 1980. Noradrenergic innervation of the rat hypothalamus:experimental biochemical and electron microscopic studies. Brain Res. 191, 161–171.
Paxinos, G., Watson, C., 2005. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.
Perry, J.A., Olver, C.S., Burnett, R.C., Avery, A.C., 2005. Cutting edge: the acquisition of TLR tolerance during malaria infection impacts T cell activation. J. Immunol. 174, 5921–5925.
Piotrowski, J., Jedrzejewski, T., Kozak, W., 2014. Heme oxygenase-1 induction by cobalt protoporphyrin enhances fever and inhibits pyrogenic tolerance to lipopolysaccharide. J. Therm. Biol. 45, 69–74.
Raffaini, M.S., Dias, M.B., Branco, L.G., 2006. Central heme oxygenase-carbon monoxide pathway participates in the lipopolysaccharide-induced tolerance in rats. Brain Res. 1111, 83–89.
Roth, J., Blatteis, C.M., 2014. Mechanisms of fever production and lysis: lessons from experimental LPS fever. Compr. Physiol. 4, 1563–1604.
Saper, C.B., Romanovsky, A.A., Scammell, T.E., 2012. Neural circuitry engaged by prostaglandins during the sickness syndrome. Nat. Neurosci. 15, 1088–1095.
Saramago, E.A., Borges, G.S., Singolani Jr., C.G., Nogueira, J.E., Soriano, R.N., Carnio, E.C., Branco, L.G.S., 2019. Molecular hydrogen potentiates hypothermia and prevents hypotension and fever in LPS-induced systemic inflammation. Brain Behav. Immun. 75, 119–128.
Soriano, R.N., Branco, L.G., 2010. Reduced stress fever is accompanied by increased glucocorticoids and reduced PGE2 in adult rats exposed to endotoxin as neonates. J. Neuroimmunol. 225, 77–81.
Steiner, A.A., Branco, L.G., 2002. Role of the preoptic carbon monoxide pathway in endotoxin fever in rats. Brain Res. 927, 27–34.
Tkacs, N.C., Strack, A.M., 1995. Systemic endotoxin induces Fos-like immunoreactivity in rat spinal sympathetic regions. J. Auton. Nerv. Syst. 51, 1–7.
Tracey, K.J., 2002. The inflammatory reflex. Nature 420, 853–859.
Vida, G., Pena, G., Deitch, E.A., Ulloa, L., 2011. alpha7-cholinergic receptor mediates vagal induction of splenic norepinephrine. J. Immunol. 186, 4340–4346.
Villanueva, A., Yilmaz, S.M., Millington, W.R., Cutrera, R.A., Stouffer, D.G., Parsons, L.H., Cheer, J.F., Feleder, C., 2009. Central cannabinoid 1 receptor antagonist administration prevents endotoxic hypotension affecting norepinephrine release in the preoptic anterior hypothalamic area. Shock 32, 614–620.