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Baroreflex activation in conscious rats modulates the joint inflammatory response via sympathetic function

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ABSTRACT

The baroreflex is a critical physiological mechanism controlling cardiovascular function by modulating both the sympathetic and parasympathetic activities. Here, we report that electrical activation of the baroreflex attenuates joint inflammation in experimental arthritis induced by the administration of zymosan into the femorotibial cavity. Baroreflex activation combined with lumbar sympathectomy, adrenalectomy, celiac subdiaphragmatic vagotomy or splenectomy dissected the mechanisms involved in the inflammatory modulation, highlighting the role played by sympathetic inhibition in the attenuation of joint inflammation. From the immunological standpoint, baroreflex activation attenuates neutrophil migration and the synovial levels of inflammatory cytokines including TNF, IL-1β and IL-6, but does not affect the levels of the anti-inflammatory cytokine IL-10. The anti-inflammatory effects of the baroreflex system are not mediated by IL-10, the vagus nerve, adrenal glands or the spleen, but by the inhibition of the sympathetic drive to the knee. These results reveal a novel physiological neuronal network controlling peripheral local inflammation.

1. Introduction

The nervous system senses peripheral inflammation triggering behavioral and cardiovascular physiological responses to maintain physiological homeostasis (Borovikova et al., 2000; Tracey, 2002; Maier and Watkins, 2003; Volman et al., 2005; Bassi et al., 2012). Clinical and experimental studies revealed that alterations of the autonomic nervous system associate with peripheral and chronic inflammatory disorders (Mravec, 2007; Stojanovich, 2009; Pongratz and Straub, 2013; Zubcevic et al., 2014). Recent studies from Tracey and coworkers have defined the inflammatory reflex, as a "*mechanism by which the nervous system reflexively regulates the inflammatory response in real time, just as it controls heart rate and other vital functions*" (Tracey, 2002; Ulloa, 2005). These studies reveal that the efferent neural-to-immune arm of this reflex system – is mediated by the activation of the vagus nerve (Tracey, 2002; Huston et al., 2006; Rosas-Ballina and Tracey, 2009). This nerve is the main component of the parasympathetic nervous system and its activation reduces cytokine production in the spleen (Rosas-Ballina and Tracey, 2009). On the other hand, the sympathetic nervous system can also regulate the innate immune system (Katafuchi et al., 1993; Kox et al., 2014; Martelli et al., 2014). Patients with chronic rheumatoid arthritis exhibit autonomic dysfunctions characterized by reduced parasympathetic and increased sympathetic tone (Pongratz and Straub, 2013), these effects associated with increased plasma levels of inflammatory cytokines such as TNF, IL-1β, and IL-6 (Tetta et al., 1990; Danis et al., 1992). Furthermore, a high sympathetic activity is linked to severe clinical signs and increased peripheral inflammation in rheumatoid arthritis (Hürlimann et al., 2002; Huston and Tracey, 2011; Pongratz and Straub, 2013), suggesting that low sympathetic activity could ameliorate inflammation in rheumatoid arthritis. Together, these studies indicate that both the parasympathetic and sympathetic nervous systems can control systemic inflammation.

Cardiovascular homeostasis is essential for the survival of humans and the maintenance of blood perfusion and oxygenation of vital organs (Abboud and Benson, 2015). The baroreflex is a critical physiological mechanism controlling cardiovascular

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homeostasis (Di Rienzo et al., 2001) by inhibiting the sympathetic outflow and increasing the parasympathetic drive, to control the arterial pressure and heart rate. The baroreceptors are mechanoreceptor sensory neurons located in the aortic arch, carotid sinuses, and major blood vessels that monitor the arterial pressure. These neurons can promote a neural response inhibiting the sympathetic activity, and increasing the parasympathetic tone (Krieger et al., 1982; Chapleau et al., 1988). Several studies demonstrated that hypertensive subjects have a higher inflammatory profile (Khraibi, 1991; Dzielak, 1992; Fu, 1995; Shen et al., 1995; Suzuki et al., 1995) combined with the attenuation of baroreflex function (Ferguson et al., 1984; Gronda et al., 2014). Therefore, it is conceivable that the higher inflammatory profile is linked to baroreflex dysfunction in arterial hypertension.

Taking into account that anesthesia attenuates baroreflex function (Palmisano et al., 1991; Tanaka and Nishikawa, 1999; Akine et al., 2001; Bassani et al., 2013), our laboratory developed a technique to electrically activate the aortic depressor nerve, an afference of the baroreflex, in conscious rats. We demonstrated that electrical stimulation of the aortic depressor nerve activates the baroreflex promoting bradycardia and hypotension, under physiological (De Paula et al., 1999; Durand et al., 2009) and pathophysiological conditions (Salgado et al., 2007; Durand et al., 2009, 2012), without the undesirable effects of anesthesia. Moreover, electrical stimulation of the aortic depressor nerve in conscious rats shifts the sympathovagal balance toward a parasympathetic predominance (Krieger et al., 1982; Chapleau et al., 1988).

Therefore, we investigated whether the activation of the baroreflex, by electrical stimulation of the aortic depressor nerve in conscious rats, controls peripheral inflammation in experimental arthritis. Furthermore, we also investigated the mechanisms mediating baroreflex control of peripheral inflammation by performing lumbar sympathectomy (the knee joint receives only sympathetic innervation) or celiac subdiaphragmatic vagotomy to distinguish the role played by sympathetic and parasympathetic efferences, respectively, and adrenalectomy or splenectomy to preclude a role played by these organs during the baroreflex activation.

2. Materials and methods

The experimental protocols comply with the recommendations of the SBNeC (Brazilian Society of Neuroscience and Behavior), the Ethical Principles of the Brazilian College of Animal Experimentation (COBEA Protocol 137/2013), and the US National Institutes of Health Guide for The Care and Use of Laboratory Animals.

2.1. Animal experiments

Male Wistar rats (250–300 g), obtained from the Main Animal Facility of the Medical School of Ribeirão Preto, University of São Paulo, were housed upon arrival at the animal facility in plastic cages under a 12-h light/dark cycle (lights on at 7 am) at 20 °C ± 1 °C and maintained in groups of five per cage (40 × 33 × 18 cm). The animals had unrestricted access to food and tap water. The number of animals used was the minimum required to ensure reliability of the results, and every effort was made to minimize animal discomfort.

2.2. Surgical procedures

Animals were anesthetized with a ketamine and xylazine mixture (50 mg/kg and 10 mg/kg) administered into the right posterior calf muscle. After the confirmation of a surgical plane of anesthesia by lack of response to a foot pinch, animals were maintained in supine position, and a medial laparotomy was performed, while one of the following procedures was applied:

(1) Splenectomy (SPLENX): the spleen was removed after ligation of the splenic blood vessels.
(2) Sympathectomy (SYMPX): the right lumbar sympathetic ganglia (L2–L3 level) were dissected near the renal artery; the L5 ganglion was identified at the level of aorta bifurcation, and all pathways connecting L2–L5 were excised.
(3) Subdiaphragmatic vagotomy (VAGX): the posterior wall of the esophagus was visualized to show the celiac branch of the vagus nerve, the celiac branch was followed until its exit from the esophageal hiatus and then 1–2 mm length of the nerve was removed.
(4) Bilateral adrenalectomy (ADX) was performed through a dorsal approach; to avoid body electrolyte loss, rats were provided with free access to 0.9% NaCl.

At the end of surgery, all animals received an injection of a polyvalent veterinary antibiotic (Pentabiótico, 0.2 mL, intramuscular; Fort Dodge, Campinas, SP, Brazil) and an injection of the anti-inflammatory and analgesic flunixin meglumine (Banamine, 25 mg/kg, subcutaneous; Schering-Plough, Cotia, SP, Brazil). A post-surgery period of 7–10 days elapsed until the implantation of the electrodes into the aortic depressor nerve. No significant postoperative weight loss or mortality was observed in the animals.

2.3. Electrode implantation

Animals were anesthetized with a cocktail of ketamine and xylazine (50 mg/kg and 10 mg/kg, i.p.) and subjected to a ventral neck surgery under microscope to isolate the left aortic depressor nerve below its joint to the superior laryngeal nerve. The aortic depressor nerve was implanted with a bipolar stainless steel electrode with an inter-leads distance of 2 mm. The electrodes were constructed by attaching two 40 mm-long stainless-steel wires (0.008 inches bare, 0.011 inches Teflon coated; model 791400; A-M Systems, Sequim, WA, USA) to a small plug (GF-6; Microtech, Boothwyn, PA, USA). The bared tips of the electrodes consisted of 2 mm lengths, forming hooks that were implanted around the aortic depressor nerve. First, the electrode was tunneled through the sternocleidomastoid muscle and the small plug was exteriorized in the nape of the neck. Next, the short segment of the aortic depressor nerve that was implanted with the bipolar stainless steel electrodes was carefully covered with silicone impression material (Kwik-Sil silicone elastomer; World Precision Instruments, Sarasota, FL, USA). The sham group underwent similar surgical procedures but was not subjected to electrical stimulation of the aortic depressor nerve. Under the same anesthesia, the left carotid artery was catheterized with polyethylene tubing (PE-50; Becton Dickinson, Sparks, MD) for recording the pulsatile arterial pressure (PAP). The catheter was tunneled subcutaneously, exteriorized in the nape of the neck and sutures closed the surgical incision sites. Flunixin meglumine (Banamine, 25 mg/kg, subcutaneous; Schering-Plough, Cotia, SP, Brazil) was injected immediately after the end of surgery.

2.4. Electrical stimulation of the aortic depressor nerve

Twenty-four hours after electrode implantation into the aortic depressor nerve, the awakened rats had the PAP recorded. In brief, the arterial catheter was connected to a pressure transducer (MLT844; ADInstruments, Bella Vista, Australia) and signal was amplified (ML224; ADInstruments, Bella Vista, Australia) and sampled by a IBM/PC computer (Core 2 duo, 2.2 GHz, 4 GB ram)

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