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Modulation of experimental arthritis by vagal sensory and central brain stimulation

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ABSTRACT

Articular inflammation is a major clinical burden in multiple inflammatory diseases, especially in rheumatoid arthritis. Biological anti-rheumatic drug therapies are expensive and increase the risk of systemic immunosuppression, infections, and malignancies. Here, we report that vagus nerve stimulation controls arthritic joint inflammation by inducing local regulation of innate immune response. Most of the previous studies of neuromodulation focused on vagal regulation of inflammation via the efferent peripheral pathway toward the viscera. Here, we report that vagal stimulation modulates arthritic joint inflammation through a novel "afferent" pathway mediated by the locus coeruleus (LC) of the central nervous system. Afferent vagal stimulation activates two sympatho-excitatory brain areas: the paraventricular hypothalamic nucleus (PVN) and the LC. The integrity of the LC, but not that of the PVN, is critical for vagal control of arthritic joint inflammation. Afferent vagal stimulation suppresses articular inflammation in the ipsilateral, but not in the contralateral knee to the hemispheric LC lesion. Central stimulation is followed by subsequent activation of joint sympathetic nerve terminals inducing articular norepinephrine release. Selective adrenergic beta-blockers prevent the effects of articular norepinephrine and thereby abrogate vagal control of arthritic joint inflammation. These results reveals a novel neuro-immune brain map with afferent vagal signals controlling side-specific articular inflammation through specific inflammatory-processing brain centers and joint sympathetic innervations.

1. Introduction

Articular inflammation is the most typical hallmark and major pathological burden in clinical and experimental arthropathies such as rheumatoid arthritis. Articular inflammation causes joint pain, edema, stiffness as well as loss of functionality due to the infiltration of leukocytes into the synovial cavity and the production of inflammatory cytokines such as tumor necrosis factor (TNF) (Firestein, 2003; Sweeney and Firestein, 2004). Neutrophils are the first type of leukocytes that migrate to the trauma site to eliminate infectious agents and to perform tissue clearance (Firestein, 2003; Sweeney and Firestein, 2004). However, unregulated neutrophilic activity causes joint deformities and motor disabilities as observed in rheumatoid arthritis (Mantovani et al., 2011; Wright et al., 2014). Large amounts of neutrophils are found in the synovial fluid of both clinical and experimental arthritic joint inflammation, especially in the early phases (Firestein, 2003; Kolaczkowska and Kubes, 2013; Mohr et al., 1981; Sweeney and Firestein, 2004; Wright et al., 2014). Currently, there is no cure for rheumatoid arthritis and the best available clinical treatments are based on the use of new biological disease-modifying anti-rheumatic drugs (bDMARDs) that act mainly by neutralizing TNF and preventing neutrophil activation (Edrees et al., 2005; Inui

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and Koike, 2016; Mantovani et al., 2011; Upchurch and Kay, 2012; Wright et al., 2014). These new treatments are still expensive and can increase the risk of infections, malignancies, and immunosuppression (Favalli et al., 2009; Inanc and Direskeneli, 2006; Smitten et al., 2008). Thus, recent experimental efforts focus on the local control of articular inflammation in order to avoid the systemic side-effects of conventional pharmacological therapies. Here, we report that vagal stimulation controls arthritic joint inflammation by inducing a local neuroimmune pathway.

The regulation of immunity by the nervous system has been widely studied, and the discovery of these mechanisms helped to design novel therapeutic strategies for inflammatory diseases (Ordovas-Montanes et al., 2015; Ulloa, 2005). Among these neuroimmune pathways, vagus nerve stimulation (VNS) has received most of the attention due to its potential to control inflammation and improve survival in experimental models of infectious and inflammatory disorders (Borovikova et al., 2000; Koopman et al., 2016; Levine et al., 2014; Matteoli et al., 2013; Wang et al., 2004). These studies reported that electrical vagal stimulation regulates peripheral inflammation through an efferent peripheral pathway mediated by the sympathetic splenic nerve, splenic lymphocytes producing acetylcholine, and α7-nicotinic acetylcholine receptors (α7nAChR) modulating macrophages (Olofsson et al., 2012). However, different investigators have suggested that, in addition to this efferent pathway, vagal stimulation may also trigger afferent signals toward the brain that may contribute to modulate the immune system (Bratton et al., 2012; Cano et al., 2001; Inoue et al., 2016; Martelli et al., 2014; Olofsson et al., 2015; Vida et al., 2011). For example, afferent vagal stimulation decreased bradykinin-induced plasma extravasation by modulating the sympatho-adrenal system (Miao et al., 1997a,b). We also noticed that stimulation of the intact (efferent and afferent) vagus nerve reduced systemic inflammation in α7nAChR-deficient mice. By contrast, specific efferent stimulation of the distal vagal trunk of the sectioned vagus nerve failed to control systemic inflammation in α7nAChR-deficient mice (Vida et al., 2011). In addition, VNS protected the kidneys against inflammation-induced injury even when the contralateral vagus nerve was blocked by local anaesthetic inhibiting efferent signals (Inoue et al., 2016). All these studies suggest the existence of an afferent vagal pathway regulating peripheral inflammation via the central nervous system. Here, we report a new central neuroimmune pathway that induces local control of articular inflammation. This novel neuroimmune network reveals specific sympatho-excitatory brain structures regulating local sympathetic components to control arthritic joint inflammation.

2. Material and methods

2.1. Animal experiments

Male Wistar rats (250–300 g), male Swiss and Swiss nude (20–24 g), C57 wild type and C57 TRPV1 KO mice were obtained from the main Animal Facility of the Ribeirão Preto Medical School, University of São Paulo, and housed upon arrival at the animal facility in plastic cages under a 12-h light/dark cycle (lights on at 7am) at 20 °C ± 1 °C. 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. All animals were anesthetized with a mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg, respectively) administered into the right posterior calf muscle through a 30G needle. 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 Protocols 137/2013, 189/2015) and the US National Institutes of Health Guide for The Care and Use of Laboratory Animals.

2.2. Surgical procedures

After the confirmation of anesthesia by the lack of response to a foot pinch, rats were maintained in supine position, and a medial laparotomy was performed, and one of the following surgical procedures was done: *splenectomy (SPX)*, the spleen was visualized, exposed and then removed after ligation of all splenic blood vessels; *subdiaphragmatic vagotomy (sVNX)*, the posterior wall of the oesophagus was visualized to find the posterior vagal branch, which was followed until its exit from the oesophageal hiatus, and then 1–2 mm length of the nerve was removed; *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 to L5 were excised as we previously described in Bassi et al. (2015); *adrenalectomy (ADX)* was performed after bilateral dorsal incision followed by visualization of the kidneys, both adrenal glands were then removed. Adrenalectomized animals had free access to 0.9% NaCl to avoid body electrolyte loss. *Cervical vagotomy (cVNX)* was performed through a ventral neck approach; the left vagus nerve was dissected from the carotid artery and cut. After each surgery, the wounds were carefully closed with sutures using nylon thread. Experiments were performed 7–10 days after the surgeries.

2.3. Drug administration

All drugs were purchased from Sigma-Aldrich® (Saint Louis, MO, USA) and dissolved in sterile saline solution. The injections were performed in animals under anesthesia and administered through a 30G needle. Different drugs were administered to the animals, alone or in combination, according to the following chronology: (a) Propranolol (5 $mg.kg^{-1}$/100 μL) was injected into the penile vein 10 min before VNS; (b) Guanethidine (0.3 μg) was injected into the femorotibial joint 48 and 24 h before VNS; (c) Butoxamine (0.3 μg) or atenolol (30 μg) were injected into the femorotibial joint 10 min before VNS; (d) Norepinephrine (NA), procaterol or dobutamine were injected into the femorotibial joint 10 min before the knee zymosan injection; (e) Lidocaine devoid of vasoconstrictors (20 $mg.mL^{-1}$ – Xylestesin®, Cristália, SP, Brazil) was injected (10 μL) inside the vagus nerve's perineurium just before its entrance under the sternocleidomastoid muscle and 3–5 mm away from the electrodes tip 5 min before vagal stimulation; (f) Cobalt chloride (1 mM $CoCl_2$ / 0.2 μL), a synaptic transmission blocker (Sandkuhler et al., 1987), was injected 10 min before vagal stimulation into the LC or PVN, through a silica capillary tube (o.d. 150 μm, i.d. 75 μm; Cluzeau Info Lab, France) and infused through an infusion pump (260; Stoelting, Wood Dalec, IL, USA) at the rate of 0.1 $\mu L.min^{-1}$. The microinjection of cobalt in brain areas has been previously used for functional inactivation (Kretz, 1984) due its property to inhibit synaptic neurotransmission by blocking pre-synaptic calcium channels (Hagiwara and Byerly, 1981). Cobalt blocks only synaptic neurotransmission, while lidocaine blocks both synaptic transmission and the action potential of passage fibers (Sandkuhler et al., 1987). The range of the doses of fMLP, LTB4 or TNF was selected based on dose-effect curves (Supplementary Fig. 2A,C&E, respectively). All the reagents were dissolved or suspended in sterile saline. Control animals received equal volumes of sterile saline (vehicle) through the same route. A total volume of 50 μL was allowed into the femorotibial joint of rats.