European Journal of Pharmacology 792 (2016) 54–62

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

The effect of inflammation on sympathetic nerve mediated contractions in rat isolated caudal artery

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ARTICLE INFO

Keywords:
Inflammation
Complete Freund's adjuvant
Caudal artery
Alpha$_1$ and alpha$_{2B}$ adrenergic receptors
Sympathetic nerves
Neurogenic contractions

ABSTRACT

Chronic inflammatory process(es) contributes to changes in vascular function in a variety of diseases. Sympathetic nerve-mediated responses in blood vessels play a pivotal role in regular physiological functions. We tested the hypothesis that sympathetic neuro-effector function will be altered as consequence of inflammatory state. Sympathetic nerve-mediated contractions and alpha adrenergic receptor expressions were evaluated in isolated caudal arteries of rats treated with saline and Complete Freund's adjuvant (CFA). While CFA-treated animals had significantly higher plasma levels of tumor necrosis factor-alpha compared to saline, blood pressure remained unchanged. Immunofluorescence revealed increased expression of ionized calcium adapter binding molecule-1 in the adventitia of blood vessels from CFA-treated animals compared to saline. In isolated arteries, electrical field stimulations between 1.25 and 40 Hz resulted in frequency-dependent contractions that wasabolished by tetrodotoxin. Neurogenic contractions from CFA groups were significantly greater than saline. While the presence of alpha$_1$-adrenoceptor antagonist (prazosin) significantly inhibited contractions at lower frequencies of stimulation (1.25–5 Hz) in isolated arteries of CFA-treated rats compared to controls, alpha$_2$-adrenoceptor antagonist (rauwolscine) had modest effects. Inhibition of neuronal reuptake by cocaine comparably enhanced field-stimulated responses in vessels of experimental and control animals. Immunofluorescence revealed a difference in expression of alpha$_1$- and alpha$_2$-adrenoceptors in the endothelium of blood vessels of CFA compared to saline controls. Collectively, our observations lend support to enhanced neurogenic contractions in blood vessels of inflamed animals possibly attributing to alterations in responsiveness and/or distribution of post-junctional alpha$_1$-adrenoceptors.

1. Introduction

Sympathetic nervous system (SNS) is a critical component of controlling vascular tone and plays a pivotal role in regular physiological functions. Pathophysiological conditions leading to the disruption of physiological function may alter SNS ability to maintain vascular homeostasis. Inflammation can also affect normal physiological function at many levels within the system, even when initiated as protective mechanism. Controlled inflammation is necessary for immunological function as immune regulatory cells are constantly interacting with foreign bodies in the system to regulate pro-inflammatory effector cells (Jiminez et al., 2015). However, excessive and persistent inflammation characterizes a variety of vascular dysfunctions. Noted changes include increases in the expression of adhesion molecules, endothelial cell damage, and activation of pro-inflammatory cytokines (Collie-Duguid and Wahle, 1996; Dinarello, 1996; Hirata et al., 1998). Among the characteristic changes during inflammation are cytokine-induced adhesion of leukocytes to endothelial cells in vasculature (Zeng et al., 2002). Chronic inflammation also appears to induce vascular endothelial and smooth muscle cell remodeling including collagen deposition, leading to altered vascular function (Lee et al., 2015).

A close interaction between the process of inflammation and sympathetic nerve-mediated function also exists, and is evident in sensory and sympathetic nerve fibers that undergo sprouting in arthritic joints (Jimenez-Andrade and Mantyh, 2012). Sympathetic fiber sprouting in inflamed joints and adjacent skin have also been reported (Longo et al., 2013). Evidence of the specific roles of adrenergic receptors within the inflammatory process indicates its activation contributes to inflammatory response (Schaible and Straub, 2014), and is also involved in activating pro-inflammatory mediators (Pongratz and Straub, 2014). The pro-inflammatory cytokines themselves have been found to also act at the central nervous system to drive

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http://dx.doi.org/10.1016/j.ejphar.2016.10.030
Received 18 July 2016; Received in revised form 19 October 2016; Accepted 21 October 2016
Available online 25 October 2016

sympathetic nerve activity (Wei et al., 2015). Clinical evidence in literature supports an interaction between the autonomic nervous system and the immune system and this appears to play a pivotal role in cardiovascular diseases (Singh et al., 2014) such as hypertension (Grassi et al., 2015). Accordingly, chronic activation of the SNS appears to change the function of the immune cells and contribute to the hypertrophy and fibrosis of the heart (Levick et al., 2010). In diseases such as autoimmune arthritis, there is evidence of a direct effect of the SNS in influencing the immune cells and the severity of inflammation. This involvement has led to catecholamine-producing cells and tyrosine hydroxylase-positive cells of the synovial tissue being targeted as new therapeutic sites of treatment in arthritis (Capellino et al., 2010; Jenei-Lanzl et al., 2015).

The rat caudal artery is densely innervated with noradrenergic nerve plexus (Jobling and McLachlan, 1992; Sittiracha et al., 1987). The sympathetic-mediated neuro-effector function has therefore been extensively studied in this artery, including the responsiveness of α-adrenoceptor-mediated contractions in normal and disease states (Aqel et al., 1986; Gisbert et al., 2002; Guimaraes et al., 1995; Seasholtz et al., 2001; Tripovic et al., 2013, 2010). α-Adrenoceptors are recognized to be responsible for mediating the effects of neuronally released norepinephrine in the caudal artery (Sulpizio and Hieble, 1991; Tanaka et al., 2004). Further evidence indicates different α_1-adrenoceptor subtypes (e.g., α_{1A}, α_{1B}, & α_{1D}) are responsible for mediating contractions (Jahnichen et al., 2004; Kamikihara et al., 2007, 2005). In addition, sympathetic-nerve mediated contractions have been determined to occur via activation of α_1- but not α_2-adrenoceptors under normal conditions (Brock et al., 1997; Jobling et al., 1992). However, to our knowledge, there is currently no evidence of α-adrenoceptor expressions reported in the rat caudal artery using immunofluorescence.

In this investigation, we tested the hypothesis that alteration to sympathetic neuro-effector function could occur in a state of chronic inflammation. Thus we studied the effects of nerve-mediated contractions as well as characterized the expressions of α_1-and α_{2b}-adrenoceptors in isolated rat caudal arteries from chronically inflamed and control animals.

2. Materials and methods

2.1. Animal model- chronic (systemic) inflammation

All procedures on animals were performed in accordance with the guidelines and regulations of the Canadian Council on Animal Care, with the approval of the Institutional Animal Care Committee of Memorial University of Newfoundland. Male Sprague Dawley rats were purchased (200–300 g; Charles River, Canada) and acclimatized for one week in temperature-controlled plastic ventilated cages. Systemic inflammation was induced using similar methods as Schopf et al. (2006). Briefly, animals were anaesthetized with isofluorane and either Complete Freund's adjuvant (CFA; 0.05 ml of a suspension of *Mycobacterium butyricum* ([10 mg/ml] in incomplete Freund's adjuvant (Sigma, USA)) or saline (equi-volume) was injected intradermally in the hind left footpad of treatment animals. Buprenorphine (0.03 mg/kg) was administered subcutaneously immediately following surgery and at q12h in CFA-treated animals for three weeks.

2.2. Blood pressure

Blood pressure was recorded weekly (for three weeks) by tail-cuff plethysmography (Model 59, IITC Inc., Woodland Hills, CA, USA). Four sequential readings were taken at 4 s apart per rat at each time point and the mean was recorded. The percentage change from baseline of systolic blood pressure was calculated.

2.3. Plasma samples and TNF α analysis

Blood samples from the left or right caudal tail vein (0.5–0.7 ml/sampling) were collected using heparin-coated syringes at baseline and for three consecutive weeks (n=5/experimental group). Samples were spun and the collected plasma was stored in a −80 °C freezer until analysis. Systemic levels of TNF α within the experimental period were performed using enzyme-linked immunosorbent assay (ELISA) kit purchased from Biolegend (San Diego, CA, USA).

2.4. Body weight and assessment of inflammation

Body weight, ankle, and paw swelling were measured in 2 day intervals, starting at Day 0 for three weeks. A weight loss of more than 20%, as well as decline of mobility, ulceration, and severe dehydration were considered endpoint of the experiment. Hock and paw widths of both hind paws were determined using digital caliper, and swelling was measured using water displacement of the ipsilateral paw and of ankle. Values were calculated as an average of three data points.

2.5. Blood vessel preparation and tension measurements

Following the three week monitoring period, animals were sacrificed by administering of 50:10 mg/kg ketamine: xylazine intraperitoneally and exsanguinated. The proximal end of the ventral caudal arteries was then isolated, and helical strips (segments approximately 1.5–2 cm) were dissected in Krebs buffer ((mM): 130 NaCl, 4 KCl, 1.2 $MgCl_2{\cdot}6H_2O$, 2.5 $CaCl_2{\cdot}2H_2O$, 12.5 $NaHCO_3$, 1.2 KH_2PO_4 and 0.1 EDTA). The buffer was previously prepared and gassed with a mixture of 95% O_2:5% CO_2 with pH 7.3–7.4, and maintained at 37 ± 1 °C in a water bath. A solution of high potassium (80 mM) was also prepared where equimolar levels of sodium ions were replaced with potassium ions. The strips were then mounted on holders with rings of platinum electrodes, connected to a force displacement transducer in an electric field stimulation system as described by Duggan et al. (2011). Vascular contractile responses were recorded on a PC-based data acquisition system, Acknowledge 3.9.1 (Biopic Systems Canada Inc.).

2.6. Electric field stimulation

Contractile responses of tissues to electric-field stimulation were measured after the tissues were subjected to a resting preload tension of 200 mg for 30–40 min (pre-determined by pilot studies of force-tension curves to 1.0 μm phenylephrine). Effect of pharmacological agents to field-stimulated contractions at various frequencies were investigated. Tissues were washed with Krebs buffer after each stimulation block and allowed to equilibrate for 20–30 min before the next treatment. Sympathetic nerves around the caudal arteries were electric field stimulated, and frequency-dependent contractile responses were taken at stimulations of varying frequencies (1.25–40 Hz). A Grass stimulator (Model S88) delivered electricity (100 mA, 50 V, 0.5 ms) through the platinum electrode rings on the tissue holders. After equilibration, the tissues underwent up to three blocks of field stimulation in the absence and presence of a pharmacological agents and/or vehicle. Tissues were assigned to pharmacological treatments on an ad hoc basis. A total of eight experimental groups were determined with tissues from CFA or saline-treated animals. They consisted of vehicle (twice distilled water 60 μl), prazosin (0.3 μm), rauwolscine (3.0 μm), WB 4101 (0.03 μm), cocaine (1.0 μm) and a combination of cocaine and each of α-adrenoceptor antagonists. The neurogenic origin of the contractile responses with field stimulations was also verified by addition of tetrodotoxin (0.1 μm) to the bath. A contraction was induced with a solution of high potassium (80 mM) to normalize the electrical field evoked responses in each individual blood vessel preparation. All measurements for the electric field-evoked contractions (area under the mechanogram) were