International Journal of Cardiology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Mast cells modulate the pathogenesis of leptin-induced left stellate ganglion activation in canines

Yuhong Wang [1], Lilei Yu [1], Guannan Meng, Zhenya Wang, Zhen Zhou, Yifeng Zhang, Hao Xia *, Hong Jiang *

Department of Cardiology, Renmin Hospital of Wuhan University, Cardiovascular Research Institute, Wuhan University, Hubei Key Laboratory of Cardiology, Wuhan, Hubei, PR China

ARTICLE INFO

Article history:
Received 27 May 2018
Received in revised form 9 July 2018
Accepted 24 July 2018
Available online xxxx

Keywords:
Mast cell
Leptin
Left stellate ganglion
Obesity
Arrhythmia

ABSTRACT

Background: Leptin is an adipocytokine predominantly secreted by adipose tissue that participates in immune modulation. Mast cells are important immune cells that are related to altered sympathetic activity. Previous study has shown that leptin promotes activation of the left stellate ganglion (LSG) directly via the leptin receptor. This study aims to investigate whether mast cells play a key role in indirect activation.
Methods: Twenty-eight canines were randomly divided into 3 groups: the control group (saline, n = 8), leptin group (leptin, n = 9), and DSCG group (disodium cromoglycate plus leptin, n = 11). Drugs were locally microinjected into the LSG. The function and neural activity of the LSG were evaluated to investigate LSG activation. Tryptase was adopted to identify activated mast cells in the LSG.
Results: Compared with the control group, leptin injection (18 μg) markedly increased the function and neural activity of the LSG. Leptin also upregulated c-fos, nerve growth factor (NGF), and tryptase expression in the LSG. However, these effects of leptin were attenuated by pre-injection of DSCG (25 mg). Additionally, the immunofluorescence analysis revealed that many mast cells were present in the LSG and that those cells were located close to sympathetic neurons. The presence of leptin receptors on the mast cells was verified.
Conclusions: Immune mast cells play an important supplementary role in the pathogenesis of leptin-induced LSG activation.

1. Introduction

Obesity, which is associated with an increased risk of cardiovascular diseases, has become a global epidemic. Mounting evidence has shown a dose-response relationship between obesity and arrhythmias [1]. Arrhythmias are closely related to dysfunction of the cardiac autonomic nervous system, particularly the left stellate ganglion (LSG), which plays an important role in modulating cardiac electrophysiology and arrhythmias [2–4]. Our previous study found that an enhanced level of the adipocytokine leptin promoted LSG activation and thus led to arrhythmia [5]. Leptin may be the key link between obesity and arrhythmias, possibly by enhancing the neural activity of the LSG. Direct activation of the LSG via the leptin receptor has been demonstrated [5]; however, the potential action of leptin on non-neuronal cells (i.e., mast cells) of the LSG is unclear.

Mast cells are important immune cells that are affected by a number of potential triggers and release various kinds of mediators after activation, including chemokines, cytokines, histamine, and proteases. Recent discoveries suggest that mast cells play an important role in the pathogenesis of obesity, which is a state defined by excess adipose tissue and adipocytokine disorder [6, 7]. Furthermore, mast cells have been demonstrated to be related to the adipocytokine leptin, which is an important mediator between the neuroendocrine and immune systems [8]. Mast cells have been found in close proximity to sympathetic neurons [9, 10], and the perineuronal location is particularly relevant for altered sympathetic activity [11]. In the present study, we aim to investigate the effect of leptin on mast cells and the role of these cells in leptin-induced LSG activation.

2. Methods

The experiment was approved by the Animal Care and Use Committees of Renmin Hospital of Wuhan University and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85–23, revised 1996).

2.1. Animal preparation

Twenty-eight male canines weighting 14–16 kg were included in this study. First, 3% sodium pentobarbital was used for intravenous anesthetization, which consisted of an initiation dose of 1 mL/kg, followed by a maintenance dose of 2 mL/h. After anesthetization, the dogs were artificially ventilated using a cuffed endotracheal tube. The body surface

* Corresponding authors at: Department of Cardiology, Renmin Hospital of Wuhan University, No. 238 Jiefang Road, Wuchang District, Wuhan City, Hubei Province 430060, PR China.
E-mail addresses: xiahao1966@163.com (H. Xia), whujianghong@163.com (H. Jiang).
[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.ijcard.2018.07.126
0167-5273/

electrocardiogram (ECG) and blood pressure (BP) were monitored using the Lead 7000 Lab System (Jinjiang Inc., Chengdu, China) throughout the experiment. Saline infusion (100 mL/h) was used for spontaneous fluid losses. A left thoracotomy was conducted at the fourth intercostal space of the dogs to expose the location of the LSG and the heart. The left anterior descending occlusion (LADO) was used to establish an acute ischemia model, which was performed on the left anterior descending coronary artery with 3–0 silk above the first diagonal branch. The vessel was partially occluded for 20 min and then completely tied off. Changes in the acute ST-segment and T-wave on the surface ECG confirmed the LADO.

2.2. Experimental protocol

The dogs were randomly divided into the control group (n = 8, saline injection into the LSG), the leptin group (n = 9, leptin injection into the LSG), and the DSCG group (n = 11, DSCG plus leptin injection into the LSG). Both the leptin (canine leptin, purchased from ProSpec, Rehovot Science Park, Israel) and DSCG (disodium cromoglycate or cromolyn sodium salt, purchased from Sigma, St. Louis, MO, USA) were dissolved in saline. Saline or leptin (18 μg) in a volume of 0.1 mL was slowly microinjected into the LSG at four points after thoracotomy. In the DSCG group, DSCG (25 mg) in a volume of 0.5 mL was very locally injected into the LSG at 15 min before leptin administration.

2.3. Function and neural activity of the LSG

The LSG activity was evaluated based on the LSG function and neural activity, which were measured before and 30 min after the microinjection. The LSG neural activity was also measured after acute ischemia.

The LSG function was determined as the maximal change in systolic blood pressure (SBP) in response to high-frequency electrical stimulation. The Grass-S88 stimulator (Astro-Med, West Warwick, Rhode Island, USA) delivered high-frequency electrical stimulation (20 Hz, 0.1 ms duration; level 1 = 5 to 7 V, level 2 = 8 to 10 V, level 3 = 11 to 13 V, and level 4 = 14 to 20 V), and the Lead 7000 Lab System recorded the changes in SBP. A pair of coated tungsten electrodes (2 cm in length) was inserted into the LSG surrounding fat pad for the LSG neural activity recording, and the ground lead was connected to the chest wall to reduce the noise. The recordings from LSG were made on the computer by application of a PowerLab data acquisition system (AD Instruments, New South Wales, Australia) and an amplifier (DP-304, Warner Instruments, Hamden, Connecticut, USA), which was set at 50 Hz (high-pass)-1 kHz(low-pass). Before and at 30 min after injection, a 1-min recording was determined, and then, 1-h recording was continually conducted after the LADO, which represent the baseline state, the simulated state, and the ischemia state of the LSG, respectively. Neural activity was defined as deflections with a signal: noise ratio >3:1, and the frequency and amplitude were used for the quantitative evaluation (Fig. 2D). The frequency was identified as the number of neural activity in 1 min, and the amplitude was the average in 1 min (Amplitude=$\sum$(A1 + A2 + … + An)/2n, A is the amplitude of each neural activity, n is the total number of the neural activity in 1 min). Additional details were provided in our previous study [5].

2.4. Histological staining

At the end of the experiment, the LSG tissues were excised quickly and fixed with 4% paraformaldehyde in preparation for staining. To evaluate LSG activation, c-fos and nerve growth factor (NGF) expression was detected with primary antibodies (c-fos, Servicebio, Wuhan, China; NGF, Servicebio, Wuhan, China). Tyrosine hydroxylase (TH) (Abcam, Cambridge, England) was used to reveal the location of sympathetic neurons, and tryptase (Abcam, Cambridge, England) was used as a well-known marker for mast cells. The quantitative analysis was conducted using commercially available software (Image Pro Plus, Media Cybernetics, Inc., Rockville, MD). Moreover, double staining for the leptin receptor (Lep-R) (R&D Systems, Minnesota, USA) with tryptase was used to show Lep-R expression on mast cells in the LSG. Fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) was used to locate the position of nucleus.

2.5. Statistical analysis

All data were expressed as the mean ± standard error of measurement (SEM) and were analyzed and graphed using the GraphPad Prism software 7.0 (La Jolla, CA, USA). The LSG function and neural activity were evaluated with a two-way repeated-measures ANOVA with a Bonferroni post hoc test at different time points. The histological staining results were analyzed using one-way ANOVA. The significance level was set at a p value < 0.05.

3. Results

3.1. The LSG function was increased by leptin injection and the leptin-induced increase was attenuated by DSCG pretreatment

Fig. 1A reveals the location of the LSG, which is a sympathetic ganglion housed in a nearby adipose tissue mass. Fig. 1B shows a typical image of the BP elevation induced by direct electrical stimulation of the LSG. LSG function was assessed based on the maximal change in SBP. In the control group, no significant difference was observed between baseline and 30 min after administration ($p > 0.05$), which implied the absence of time-dependent changes in BP elevation induced by LSG stimulation (Fig. 1C). Additionally, no significant difference was observed at the baseline state among the three groups ($p > 0.05$). As a result of leptin administration, the LSG function was significantly

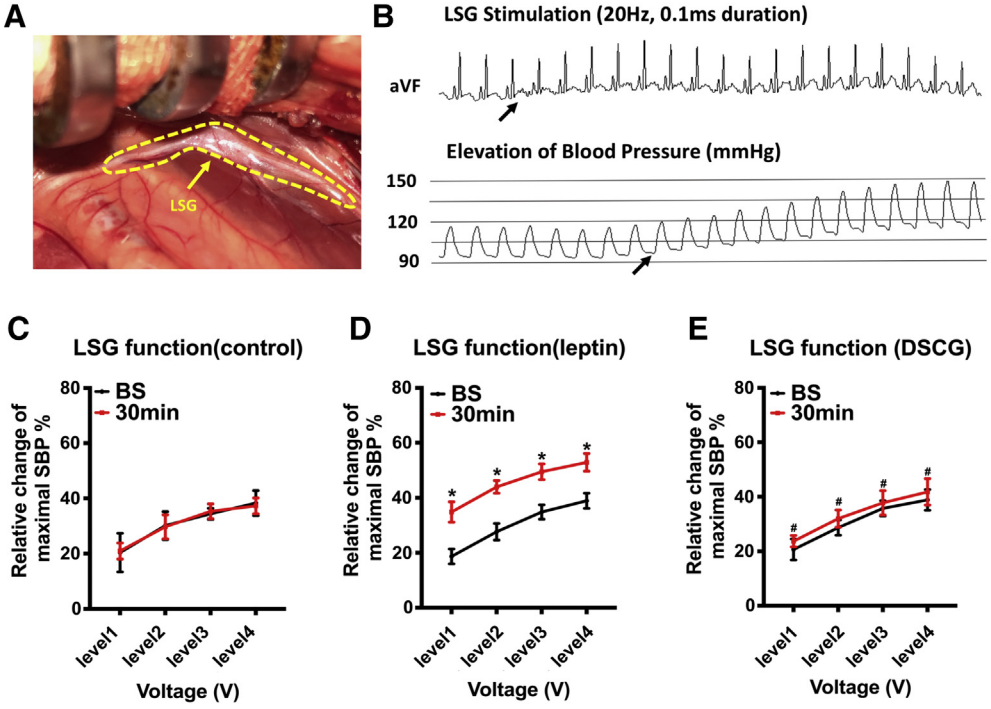

Fig. 1. Effects on LSG functions. The anatomical location of the LSG is shown in A. B is a typical image of LSG stimulation and elevation of the BP in response to LSG stimulation. Compared with the control group, leptin injection significantly increased the LSG function (*$p < 0.05$) (C–E); however, this leptin effect was attenuated by DSCG pretreatment (#$p < 0.05$) (C–E). BS, Baseline state; LSG, Left stellate ganglion; BP, Blood pressure; DSCG, Disodium cromoglycate. Level 1 = 5 to 7 V, level 2 = 8 to 10 V, level 3 = 11 to 13 V, and level 4 = 14 to 20 V.

Please cite this article as: Y. Wang, et al., Mast cells modulate the pathogenesis of leptin-induced left stellate ganglion activation in canines, Int J Cardiol (2018), https://doi.org/10.1016/j.ijcard.2018.07.126