Peptides 44 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Peptides

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

Leptin in nucleus of the solitary tract alters the cardiovascular responses to aortic baroreceptor activation

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ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 14 March 2013
Accepted 14 March 2013
Available online 25 March 2013

Keywords:
Leptin receptors
Cardiovascular reflex pathways
Aortic depressor nerve
Obesity
Leptin

ABSTRACT

Recent data suggests that neurons expressing the long form of the leptin receptor form at least two distinct groups within the caudal nucleus of the solitary tract (NTS): a group within the lateral NTS (Slt) and one within the medial (Sm) and gelantinosa (Sg) NTS. Discrete injections of leptin into Sm and Sg, a region that receives chemoreceptor input, elicit increases in arterial pressure (AP) and renal sympathetic nerve activity (RSNA). However, the effect of microinjections of leptin into Slt, a region that receives baroreceptor input is unknown. Experiments were done in the urethane-chloralose anesthetized, paralyzed and artificially ventilated Wistar or Zucker obese rat to determine leptin's effect in Slt on heart rate (HR), AP and RSNA during electrical stimulation of the aortic depressor nerve (ADN). Depressor sites within Slt were first identified by the microinjection of L-glutamate (Glu; 0.25 M; 10 nl) followed by leptin microinjections. In the Wistar rat leptin microinjection (50 ng; 20 nl) into depressor sites within the lateral Slt elicited increases in HR and RSNA, but no changes in AP. Additionally, leptin injections into Slt prior to Glu injections at the same site or to stimulation of the ADN were found to attenuate the decreases in HR, AP and RSNA to both the Glu injection and ADN stimulation. In Zucker obese rats, leptin injections into NTS depressor sites did not elicit cardiovascular responses, nor altered the cardiovascular responses elicited by stimulation of ADN. Those data suggest that leptin acts at the level of NTS to alter the activity of neurons that mediate the cardiovascular responses to activation of the aortic baroreceptor reflex.

1. Introduction

Leptin, a 16 kD protein released by adipocytes, normally functions through its central actions to decrease food intake and body weight, and increase energy expenditure by activating the sympathetic nervous system [11,19,24,33,53,69]. Released into the circulation in proportion to adiposity [36,42,70], leptin is transported through the blood brain barrier [3,4] where it binds to the long form of the leptin receptor (Ob-Rb) to activate cellular downstream pathways within neurons [3,20].

Most of leptin effects on the cardiovascular system are thought to be mediated through the activation of functional Ob-Rb that is expressed within several hypothalamic nuclei [47,57,61,62]. This is based on the observation that leptin injections into the lateral cerebral ventricles elicit transient increases in heart rate (HR), arterial pressure (AP) and renal sympathetic nervous activity (RSNA) [35,63]. Additionally, microinjections of leptin directly into the hypothalamic nuclei elicit similar increases in both AP and RSNA [47,57]. However, recent studies have also suggested that leptin exerts its effects on the cardiovascular system through direct action on caudal brainstem nuclei. The nucleus of the solitary tract (NTS), a medullary region known to be the primary site of termination of cardiovascular afferent fibers [14,16,71], has been shown to contain leptin receptors [5,18,33,34,40,50,62]. Furthermore, several studies have shown that systemic administration of leptin increases the expression of the immediate early gene c-fos within NTS [25,27]. Taken together, these findings can be interpreted to suggest that leptin exerts a direct effect at these NTS sites, possibly on neurons mediating cardiovascular reflexes. This suggestion is supported by the observation that microinjections of leptin into NTS elicit increases sympathetic activity [17,46], and attenuate the reflex bradycardia during systemic activation of the baroreflex [1]. These observations are consistent with findings that the baroreceptor reflex sensitivity is decreased in obese animals and patients with high circulating levels of leptin [23,30,32,48,57,68].

Abbreviations: 4V, 4th ventricle; 12M, hypoglossal motor nucleus; ADN, aortic depressor nerve; ANOVA, analysis of variance; ap, area postrema; AP, arterial pressure; cc, central canal; com, commissural nucleus of the NTS complex; cu, cuneate fasciculus; Cu, nucleus cuneatus; DMV, dorsal motor nucleus of the vagus; Gr, nucleus gracilis; HR, heart rate; NTS, nucleus of the solitary tract; Ob-Rb, long form of leptin receptor; RSNA, renal sympathetic nerve activity; Sg, nucleus gelantinosa (sub-postremal area) of the NTS complex; Slt, lateral nucleus of the NTS complex; Sm, medial nucleus of the NTS complex; St, solitary tract.

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0196-9781/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.peptides.2013.03.021

In a recent study that within the caudal NTS complex cells that express the Ob-Rb appear to form at least two distinct groupings: one within the medial NTS which includes the medial (Sm) and gelatinosa (Sg) subnuclei of NTS and another within the lateral (Slt) subnucleus [18]. Discrete microinjections of leptin into Sm and Sg, a region that receives direct chemoreceptor inputs [16], have been shown to elicit increases in AP and RSNA and potentiate these responses during activation of the peripheral chemoreceptor reflex [17]. On the other hand, the effect of discrete microinjections of leptin into Slt, a NTS region that receives aortic baroreceptor afferent projections [14,16] is unknown. Therefore, this study was done in the anesthetized Wistar rat and Zucker leptin receptor-deficient obese rats to investigate the effect of leptin receptor activation in NTS on the reflex cardiovascular responses to activation of aortic baroreceptors.

2. Methods and materials

2.1. General procedures for NTS injection studies

Experiments were done in 13 male Wistar and 3 adult male Zucker fatty rats (fa/fa) (250–375 g) anesthetized with a mixture of urethane and α-chloralose (1.1 g/kg and 80 mg/kg, iv, respectively) after induction with an injection of equithesin (0.3 ml/100 g, ip). Anesthesia was maintained during the course of the study with a supplementary dose of α-chloralose (10 mg/kg, iv) every hour. The animals were paralyzed with pancuronium bromide (Pavulon, Organon Canada, Toronto, ON; 1 mg/kg iv initially, and additional doses of 0.5 mg/kg iv as necessary) and artificially ventilated using a small rodent ventilator (model 683; Harvard Apparatus, Holliston, MA) with a mixture of room air and 95% O_2. During the course of the experiment, the animals were allowed to recover periodically from the paralyzing agent to determine the depth of anesthesia by examination of withdrawal and corneal reflexes. Body temperature was maintained at 36–37 °C by a heating pad (model K-20-C; American Hospital Supply Corp., Cincinnati, OH) [17]. All animals were housed under controlled conditions with a 12 h light/dark cycle. Food and water were available to all animals *ad libitum*. All experimental procedures were done in accordance with the guidelines on the use and care of laboratory animals as set by the Canadian Council on Animal Care and approved by the Animal Care Committee at The University of Western Ontario.

Polyethylene catheters (PE50; Clay Adams, Parsippany, NJ) were inserted into the right femoral artery and vein for the recording of AP and the administration of drugs, respectively. AP was recorded *via* a Statham pressure transducer (model P23 XL), and a Grass tachograph (model 7P4K) triggered by the AP pulse was used to monitor HR. AP, mean AP (MAP) and HR were recorded continuously on a Grass polygraph (model 79G) and in some cases using an ADInstruments PowerLab (model 4/25). In these latter cases, MAP and HR were calculated by ADInstruments Chart 5 Software using the AP and pulse frequency.

2.2. Identification and stimulation of the ADN

The left ADN was identified and isolated as previously described [14,60]. The nerve was dissected free for approximately 1 cm from the vagal bundle and crushed distally. The central end of the isolated ADN was placed on a bipolar stainless steel electrode, and cotton pellets soaked in Dow Corning 360 medical fluid (Dow Corning, Midland, MI) were placed around the nerve to prevent drying and to limit the spread of current to the vagus and cervical sympathetic nerves. The ADN was functionally identified by the decrease in MAP and HR elicited during electrical stimulation using a 10-s pulse train (current intensity of 0.2–1.0 mA, 25 Hz, and 0.3–0.5 ms pulse duration) [58]. In the studies where the ADN responses were interacted with those resulting from leptin injections into the ipsilateral (left) NTS, the right vagal bundle contralateral to the NTS injections sites was identified, isolated and cut.

2.3. Isolation and recording from renal nerves

A retroperitoneal dissection was used after a left flank incision, to expose the left renal nerve as previously described [12,15]. The renal nerves were identified coursing along the renal artery and vein, and separated from the blood vessels. To record RSNA, the isolated renal nerves were placed on a bipolar platinum–iridium recording electrode, and crushed distal to the electrode. The isolated nerves and the electrode were embedded in a silicone elastomer (Kwik-Sil, WPI Inc., Sarasota, FL). Additionally, cotton pellets were soaked in medical fluid (Dow Corning; Midland, MI) and placed around the nerves and other exposed tissues to prevent drying. The incision was sutured and the animal placed in a stereotaxic frame. The signal was amplified and passed through a Grass band pass filter (50–3 kHz) and viewed on an oscilloscope [12]. The output of the amplifier was connected to a Grass integrator which rectified the signal (0.5 ms time constant). The integrated and raw nerve discharges were recorded on a Grass 79 polygraph. The integrated nerve activity was then displayed as an integrated voltage that was proportional to the RSNA. The rectified signal was also recorded on a polygraph and stored on disk until analyzed using a PowerLab data acquisition system. Background noise was determined at the completion of each experiment after the systemic administration of the ganglionic blocker hexamethonium bromide (20 mg/kg, iv). This value was subtracted from the integrated values obtained for each RSNA recording. Microinjections of leptin (5–100 ng; 20 nl) [17] were made at depressor sites in Slt and the adjacent Sm of the NTS complex to determine the effect of leptin on HR, AP, and RSNA.

2.4. Microinjections into NTS

The head of the animal was placed in a Kopf stereotaxic frame and bent downwards at a 45° angle to the horizontal meridian. The dorsal surface of the medulla was exposed by partial occipital craniotomy. The dura was cut and reflected laterally, and the caudal floor of the fourth ventricle was exposed by gently removing the vermis of the cerebellum by suction. The nervous tissue was kept moist by 0.9% physiological saline throughout the experiment.

Double-barreled glass micropipettes (tip diameter, 20–35 μm) were pulled from 5 μl Socorex capillary tubing (Mississauga, ON, Canada). Micropipettes were lowered stereotaxically into the caudal left NTS region using the *calamus scriptorius* as the reference point (rostrocaudal: +0.3 to +0.5 mm; mediolateral: 0.3–0.7 mm; dorsoventral: 0.3–0.7 mm below the dorsal surface) [54]. A leptin (rat recombinant #L-5037; Sigma, St Louis, MO) stock solution was prepared according to the instructions supplied by Sigma and later diluted appropriately for use during the experiments by adding phosphate buffered saline (pH 7.2). Microinjections of leptin (5–50 ng; 20 nl) and the excitatory amino acid L-glutamic acid (Glu; 0.25 M; 10 nl; #G-1626; Sigma Chemical; St. Louis. MO) were made into NTS using the application of pressurized nitrogen pulses controlled by a picospritzer (General Valve, Fairfield, NJ). The injected volume was measured by direct observation of the fluid meniscus in the micropipette by using a microscope fitted with an ocular micrometer as previously described [17,43]. At NTS cardiovascular depressor sites in the Zucker rats, the leptin amount microinjected was increased up to 100 ng in 20 nl in an attempt to observe cardiovascular responses.

In order to investigate whether leptin modulated the cardiovascular responses induced by the microinjection of Glu into NTS, Glu