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SYSTEMIC ADMINISTRATION OF LEPTIN POTENTIATES THE RESPONSE OF NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT TO CHEMORECEPTOR ACTIVATION IN THE RAT

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Abstract—Leptin microinjections into the nucleus of the solitary tract (NTS) have been shown to elicit sympathoexcitatory responses, and potentiate the cardiovascular responses to activation of the chemoreflex. In this study, experiments were done in Sprague–Dawley rats initially to provide a detailed mapping within the NTS complex of cells containing immunoreactivity to the long form of the leptin receptor (Ob-Rb). In a second series, this NTS region containing Ob-Rb immunoreactive cells was explored for single units antidromically activated by stimulation of pressor sites in the rostral ventrolateral medulla (RVLM). These antidromically identified neurons were then tested for their response to intra-carotid injections of leptin (50–100 ng/0.1 ml), and to activation of peripheral chemoreceptors following an injection of potassium cyanide (KCN) (80 μg/0.1 ml) into the carotid artery. Cells containing Ob-Rb-like immunoreactivity were found predominantly in the caudal NTS: within the medial, commissural and gelatinous (subpostremal area) subnuclei of the NTS complex. Of 73 single units tested in these NTS regions, 48 were antidromically activated by stimulation of RVLM pressor sites and 25 of these single units responded with an increase in discharge rate after intra-carotid injections of leptin. In addition, 17 of these leptin responsive neurons were excited by the intra-carotid injections of KCN (80 μg/0.1 ml). Furthermore, the excitatory response of these single units to KCN was potentiated (59–83%) immediately following the leptin injection. These data indicate that leptin responsive neurons in NTS mediate chemoreceptor afferent information to pressor sites in the RVLM, and suggest that leptin may act as a facilitator on neuronal circuits within the NTS that potentiates the sympathoexcitatory responses elicited during the reflex activation of arterial chemoreceptors. © **2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: arterial chemoreceptors, hypertension, hypoxia, rostral ventrolateral medulla, obesity.

INTRODUCTION

Leptin, a 16 kDa protein, is normally released into the circulation by adipocytes in proportion to adiposity (Hardie et al., 1996; Jéquier, 2002; Trayhurn and Bing, 2006). Leptin within the circulation is transported through the blood–brain barrier (Banks et al., 1996; Banks, 2001) where it binds to the long form of the leptin receptor (Ob-Rb), and activates cellular downstream pathways within neurons (Banks et al., 2000; Cui et al., 2006). Within the central nervous system leptin normally acts to decrease food intake, decrease body weight and increase energy expenditure (Calapai et al., 1998; Tang-Christensen et al., 1999; Muzzin et al., 2000; Grill, 2010). The effects on energy expenditure have been shown to be due to increased sympathetic nervous activity to brown adipose tissue (Haynes et al., 1997), which is thought to arise from the activation of central mechanisms involving the hypothalamus (Dunbar et al., 1997; Correia et al., 2001; Hall et al., 2001). In support of this suggestion, intracerebroventricular administration of leptin elicits transient increases in sympathetic nervous activity, arterial pressure and heart rate (for reviews see Hall et al., 2001; Shirasaka et al., 2003). Similarly, direct microinjections of leptin into the arcuate or ventromedial hypothalamic nuclei have been shown to elicit increases in sympathetic nervous activity and arterial pressure (Marsh et al., 2003). Although both the arcuate and ventromedial hypothalamic nuclei have been shown to contain Ob-Rb (Schwartz et al., 2000; Marsh et al., 2003; Rahmouni and Morgan, 2007), there are other hypothalamic nuclei, including the paraventricular nucleus and the dorsomedial hypothalamus that also express Ob-Rb (Shioda et al., 1998; Schwartz et al., 2000), and are known to be involved in cardiovascular regulation (Dampney, 1994; Dampney et al., 2003). As a result, these findings have been interpreted to suggest that the central effects of leptin on the circulation are mediated by descending hypothalamic pathways which regulate the activity of brainstem autonomic nuclei (for reviews see Dampney, 1994; Dampney et al., 2003; Shirasaka et al., 2003).

However, there are recent studies showing that leptin can also exert its effects directly within brainstem autonomic structures. Ob-Rb have been identified in several brainstem regions, especially within the nucleus of the solitary tract (NTS) (Mercer et al., 1998; Shioda et al., 1998; Grill et al., 2002; Hosoi et al., 2002; Grill

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Abbreviations: Amb, nucleus ambiguus; ap, area postrema; Com, commissural nucleus of the NTS complex; DMV, dorsal motor nucleus of the vagus; is-sd break, initial segment-somatodendritic break; KCN, potassium cyanide; NTS, nucleus of the solitary tract; PBS, phosphate-buffered saline; pSTAT3, phospho-signal transducer and activator of transcription 3; Ob-Rb, long form of leptin receptor; RVLM, rostral ventrolateral medulla; Sm, medial nucleus of the NTS complex.

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and Hayes, 2009; Grill, 2010). The NTS is known to play a pivotal role in the reflex regulation of arterial pressure (Dampney, 1994; Spyer et al., 1997; Schreihofer and Guyenet, 2002; Dampney et al., 2003) as it is the primary site of termination of baroreceptor and chemoreceptor afferent fibers (Ciriello, 1983; Erickson and Millhorn, 1991; Finley and Katz, 1992; Mifflin, 1992; Chitravanshi et al., 1994; Ciriello et al., 1994; Chitravanshi and Sapru, 1995; Spyer et al., 1997; Dampney et al., 2003; Spyer and Gourine, 2009). These findings suggest that leptin may exert an effect on NTS neurons involved in cardiovascular reflex pathways. Of interest, several studies have reported that systemic administration of leptin increases the expression of the c-fos gene within caudal NTS (Elmquist et al., 1997, 1998; Elias et al., 2000; Grill et al., 2002), regions that are known to receive cardiovascular afferent projections (Ciriello, 1983; Erickson and Millhorn, 1991; Finley and Katz, 1992; Mifflin, 1992; Chitravanshi et al., 1994; Ciriello et al., 1994; Chitravanshi and Sapru, 1995; Spyer et al., 1997; Dampney et al., 2003; Spyer and Gourine, 2009). In addition, administration of leptin into the caudal NTS has been reported to elicit increases in arterial pressure and renal sympathetic nerve activity (Mark et al., 2009; Ciriello and Moreau, 2012). Furthermore, leptin injections into the NTS have been shown to attenuate the cardiovagal component of the baroreceptor reflex (Arnold et al., 2009), while potentiating the sympathoexcitatory responses to activation of the chemoreflex (Ciriello and Moreau, 2012). Taken together, these data suggest that there are at least two different populations of neurons within the NTS that respond to leptin; one population that is inhibited and one that is excited by leptin, and these neurons may be involved in mediating chemoreceptor or baroreceptor afferent information. *In vitro* electrophysiological data supporting this suggestion of two populations of NTS neurons that are either excited or inhibited by leptin have been reported (Yuan et al., 2000, 2002; Ellacott et al., 2006; Hisadome et al., 2010). However, which of these two populations of NTS neurons mediate the sympathoexcitatory effects is not known.

In this study, the effect of leptin on NTS neurons projecting to cardiovascular sites within the rostral ventrolateral medulla (RVLM) was investigated in the anesthetized rat. As a detailed mapping of the distribution of cells expressing the Ob-Rb in the dorsal vagal complex is not available, an immunohistochemical study was initially done to identify the location of cells within the NTS region that express the Ob-Rb (8). The NTS region containing Ob-Rb immunoreactive cells was then explored for single units antidromically activated by stimulation of pressor sites in the RVLM. These antidromically activated units were then tested for their response to an intra-arterial injection of leptin. Finally, whether the discharge rate of these neurons was also altered by peripheral chemoreceptors' activation was investigated, along with the effect of activation of chemoreceptors in combination with systemic leptin administration on the discharge rate of these NTS–RVLM projecting neurons.

EXPERIMENTAL PROCEDURES

General procedures

Experiments were done in 29 male Sprague–Dawley rats (250–375 g) anesthetized with α-chloralose and urethane mixture (80 mg/kg and 1.1 g/kg i.v., respectively) after induction with equithesin (0.3 ml/100 g, i.p.), and followed by a supplemental dose of 10 mg/kg α-chloralose (i.v.) every hour. The evening prior to the experiment laboratory rat chow was removed from the animals, although water remained available *ad libitum*. All experimental procedures were done in accordance with the guidelines on the use and care of laboratory animals as set out by the Canadian Council on Animal Care and approved by the Animal Care Committee at the University of Western Ontario.

Polyethylene catheters (PE-50) were inserted into the femoral artery and vein for the recording of arterial pressure and the administration of drugs, respectively. Arterial pressure was recorded via a Statham pressure transducer (model P23 Db; Statham-Gould, Oxnard, CA, USA), and a Grass tachograph (model 7P4FG; Grass Instrument Company, Quincy, MA, USA) that was triggered by the arterial pressure pulse was used to monitor heart rate. Both arterial pressure and heart rate were recorded continuously on a Grass polygraph (model 79D). The trachea was cannulated, the animal was paralyzed with pancuronium bromide (Pavulon, Organon Canada, Toronto, ON, Canada; 1 mg/kg i.v. initially, and additional doses of 0.5 mg/kg i.v. when necessary) and artificially ventilated using a small rodent ventilator (model 683; Harvard Apparatus, Holliston, MA, USA) 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 reflexes. Rectal temperature was monitored and maintained at 37 ± 1 °C by a heating pad controlled by a Yellow Springs temperature controller (model 73A; Yellow Springs, OH, USA).

Immunohistochemical identification of Ob-Rb expressing cells in the NTS Region

Three rats were deeply anesthetized (0.3 ml/100 g body weight, i.p.) and perfused transcardially with 300 ml of saline followed by 500 ml of Zamboni's fixative (2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2; 15% saturated picric acid). The brain was removed and then placed in Zamboni's fixative. At 24-h intervals, the brain was transferred sequentially to 5% and 10% sucrose–phosphate-buffered saline (PBS) solutions at 4 °C. Brains were then gradually dehydrated through a series of alcohols, placed in xylene followed by paraffin wax. Serial, transverse sections of the brainstem through the region of the NTS were cut on a microtome at 6 μm, mounted onto double-gelatinized glass slides and placed on a slide warmer. Tissue sections were later de-paraffinized in xylene and rehydrated using graded alcohol solutions. Sections were equilibrated using three 20-min washes of PBS (pH 7.4), and then underwent an antigen-retrieval protocol using a citrate buffer (10 mM sodium citrate/0.05% Tween; pH 6.0) heated to 90–95 °C in a microwave for 15 min (Messenger et al., 2012). Slides were rinsed and endogenous peroxidase activity was inhibited by exposing the sections to a 1% hydrogen peroxide solution for 10 min. Sections were washed in PBS and allowed to incubate overnight at 22 °C in affinity purified chicken anti-Ob-Rb (Cat # CH14104, LepRb/OBRb; Neuromics Inc., Edina,