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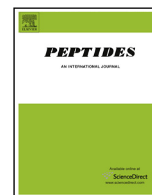

Short communication

Carotid chemoreceptor afferent projections to leptin receptor containing neurons in nucleus of the solitary tract

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ABSTRACT

Neurons expressing the leptin receptor (Ob-R) exist within the caudal nucleus of the solitary tract (NTS). Additionally, afferent neurons expressing the Ob-R have been identified within the nodose ganglion and NTS. Furthermore, systemic injections or focal injections of leptin directly into NTS potentiate the response of NTS neurons to carotid chemoreceptor activation. However, the distribution of carotid body afferents in relation to Ob-R containing neurons within NTS is not known. In this study, chemoreceptor afferent fibers were labeled following microinjection of the anterograde tract tracer biotinylated dextran amine (BDA) into the carotid body or petrosal/nodose ganglion of Wistar rats. After a survival period of 10–14 days, the NTS was processed for BDA and Ob-R immunoreactivity. Afferent axons originating in the carotid body were found to project to the lateral (Slt), gelantinosa (Sg), and medial (Sm) subnuclei of the NTS complex. A similar, but more robust distribution of BDA labeled fibers was observed in the NTS complex after injections into the petrosal/nodose ganglion. Carotid body BDA labeled fibers were observed in close apposition to Ob-R immunoreactive neurons in the region of Slt, Sg and Sm. In addition, a small number of carotid body afferents were found to contain both BDA and express Ob-R-like immunoreactivity within the regions of Slt, Sg and Sm. Taken together, these data suggest that leptin may modulate carotid chemoreceptor function not only through direct effects on NTS neurons, but also through a direct effect on carotid body primary afferent fibers that innervate NTS neurons.

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1. Introduction

Leptin, a 16-kDa peptide hormone adipocytokine produced by white adipose tissue, is released into the circulation in proportion to fat mass [13,30,34,36,38,53,59]. Leptin acts to regulate body weight and energy balance through its action on hypothalamic areas that control appetite and energy expenditure [12,59,60]. In addition, leptin elicits sympathoexcitatory effects resulting in increased arterial pressure [29,31], suggesting that overweight or obese individuals with higher circulating levels of leptin are at greater risk of developing hypertension [25,29,57]. These actions on central sympathoexcitatory systems involve the activation of both hypothalamic [41,48,55] and brainstem autonomic nuclei [16–18,32,40,55].

Within the brainstem, recent studies have shown that leptin receptors (Ob-R) are expressed predominantly within the caudal aspects of the nucleus of the solitary tract (NTS) [5,17,18,23,42,56]. Interestingly, this region of NTS is known to receive an extensive innervation from both baroreceptor and chemoreceptor afferent fibers [15,19]. The overlapping regions of Ob-R expressing neurons and cardiovascular afferent fibers within NTS indicate that leptin exerts an effect on cardiovascular reflex pathways. Leptin administration into NTS has been shown not only to inhibit baroreceptor reflex function [3,16,40], but also to potentiate the cardiovascular responses to activation of carotid chemoreceptors [17,18].

In addition to neurons expressing Ob-R within NTS, vagal afferent fibers within NTS [11,52] and nodose ganglion afferent neurons [10,11,46] have been shown to contain Ob-R. This observation suggests that the cardiovascular effects observed following injections of leptin into NTS [3,16,40] may be mediated not only by leptin's direct actions on Ob-R expressing NTS neurons, but also by exerting an effect on primary afferents fibers innervating NTS neurons by modulating the presynaptic release of neurotransmitters [45]. This study was therefore done to investigate whether chemoreceptor

Abbreviations: XII, hypoglossal nucleus; ap, area postrema; BDA, biotinylated dextran amine; cc, central canal; Com, commissural subnucleus of the NTS complex; DMV, dorsal motor nucleus of the vagus; GR, fasciculus gracilis; NTS, nucleus of the solitary tract; Ob-R, leptin receptor; PBS, phosphate buffered saline; Sg, nucleus gelatinosa (subpostremal area) of the NTS complex; Slt, lateral subnucleus of the NTS complex; Sm, medial subnucleus of the NTS complex; St, solitary tract.

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afferent fibers projecting to NTS innervate Ob-R expressing neurons and/or whether these chemoreceptor afferent fibers also express Ob-R. This was done by injecting the anterograde tract tracer BDA [8,50] into the carotid body or petrosal/nodose ganglion to identify carotid chemoreceptor afferent fibers in NTS and then combining this with immunohistochemistry for the Ob-R.

2. Methods

Experiments were done in 19 adult male Wistar rats (250–400 g; Charles River Canada). All animals were housed under controlled conditions with a 12-h light/dark cycle. 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.

On the day of the BDA injections the animals were anesthetized with equithesin (0.3 mg/100 g body weight, i.p.) [27]. The head of the animal was placed in an inverted Kopf (Tujunga, CA) stereotaxic frame and access to the carotid body ($n = 8$), carotid sinus ($n = 2$) or petrosal/nodose ganglia complex ($n = 9$) was obtained by a ventral midline incision in the neck. The carotid artery at its bifurcation was isolated and the carotid body was identified at the junction between the internal and external carotid arteries. The vagus nerve and petrosal/nodose ganglia complex were identified near the junction of the vagus and glossopharyngeal nerves and isolated from surrounding tissues. A small tray fashioned from parafilm was placed underneath the carotid body or ganglia to isolate them from the surrounding tissue and prevent inadvertent uptake of the tracer from the injection site.

Glass micropipettes (internal tip diameter 20–35 μm) were pulled from 5 μl Socorex capillary tubing and filled by capillary action with 10% BDA in 10 mM phosphate buffer (PB) (Vector Laboratories, Burlingame, CA) [4] pH 7.2–7.4. The micropipette tips were lowered into either the carotid body or petrosal/nodose ganglia complex and BDA was iontophoresed using a 5 μA cathodal current delivered through a Grass PSIU stimulus isolation unit (7 s pulses every 14 s for 30 min) [4]. Following completion of the injection, the muscles and skin overlying the carotid body and ganglia were sutured and a topical anesthetic was placed over the surgical area. All animals were given a subcutaneous injection of sterile saline (0.5 ml) and kept warm on a heating blanket at 37 °C until awake, at which time they were returned to their home cage in the animal care facility. In two animals following BDA injections into the carotid body the ipsilateral carotid sinus nerve was transected to demonstrate that the afferent labeling within NTS was due to carotid body afferents and not due to leakage of BDA into any adjacent tissue resulting in non-specific uptake of the tract tracer. Additionally, in one animal, following an injection into the petrosal/nodose ganglia complex the ipsilateral vagus and glossopharyngeal nerves were cut just rostral to the ganglia complex to determine whether the tracer had been picked up by adjacent tissues. Finally, to determine whether the BDA iontophoresed into the carotid body had gained access to the carotid sinus region, in two additional animals the BDA solution was applied directly to the carotid sinus region. Under these control conditions, no BDA labeled fibers were observed in the NTS confirming the specificity and localization of the iontophoretic BDA injection in the carotid body.

After a survival period of 10–14 days, animals were deeply anesthetized with pentobarbital sodium (65 mg/kg, i.p., Somnotol, M.T.C. Pharmaceuticals, Hamilton, Canada) and perfused transcardially with 200–300 ml 0.9% physiological saline, followed by 500 ml of fixative (4% paraformaldehyde in 0.4 M PB containing L-lysine and sodium m-periodate at pH 7.2–7.4). The brainstem was removed from the spinomedullary junction to the inferior colliculi and stored in PB saline (PBS; pH 7.2–7.4) containing 10% sucrose overnight at 4 °C.

Frozen, serial transverse sections of the brainstem were cut at 40 μm in a cryostat (−17 °C; Bright model 5030; Bright Instruments, Huntington, UK) and collected in PBS. For each rat, one in every two sections of the brainstem through the region of NTS was processed for BDA histochemistry [4] and for Ob-R immunoreactivity [18]. The adjacent sections were stained with thionin and used for the identification of cytoarchitectonic boundaries [58]. In brief, tissue sections were rinsed in PBS and first processed for BDA fluorescence followed by Ob-R immunohistochemistry. Sections processed for BDA fluorescence were rinsed in PBS and placed in 0.5 μg/ml Streptavidin Texas Red (Code: RPN1233; GE Healthcare, Buckinghamshire, England) diluted 1:100 in 0.3% Triton X-100 for 1.5 h. After rinsing in PBS the sections were then placed overnight at 22 °C in affinity purified chicken anti-Ob-R (Cat # CH14104, LepRb/OBRb; Neuromics Inc., Edina, MN, USA) antiserum (1:1000 in PBS/0.3% Triton-X 100 and 4% normal goat serum) [5,43]. This Ob-R antiserum for the long form of the leptin receptor has previously been characterized for specificity [5,43]. Following PBS washes, the sections were placed in goat biotinylated anti-chicken IgY (Vectastain Elite Kit, Vector Laboratories, Burlingame, CA) diluted 1:200 in PBS/0.3% Triton X-100 for 1 h. Sections were rinsed in PBS and placed in Streptavidin Alexafluor-488 (Cat. # S11223; Invitrogen, Burlington, ON, Canada) diluted 1:100 for 1 h. After PBS rinses, sections were placed in 100% ethanol containing 5% glacial acetic acid on dry ice for 10 min. Sections were immediately cover-glassed using Fluoromount mountant. Controls for Ob-R immunoreactivity included processing brainstem sections after the omission of the primary antibody or after preabsorption of the primary antibody with a recombinant Ob-R peptide (132.5 kDa; LepRb/OBRb, # P14014; Neuromics Inc.) [43].

BDA labeled fibers and Ob-R-like immunoreactivity within fibers and cells were identified on sections through the region of NTS using fluorescence microscopy (Leitz Diaplan; E. Leitz Wetzlar GMBH, Wetzlar, Germany). Digital images were obtained with a Nikon DS-Fil camera and NIS Elements Basic Research 3.0 software (Nikon Canada, Mississauga, ON, Canada). The location and distribution of BDA labeled and Ob-R immunoreactive fibers were also mapped onto camera lucida projection drawings of the NTS region. The atlas and nomenclature of Swanson [58] were used for the identification of medullary structures. The nomenclature of the NTS complex by Ciriello et al. [19] was also used to describe subnuclei within this structure.

3. Results

Following injections of BDA into the carotid body, labeled fibers were observed in NTS of four of the six animals with intact carotid sinus nerve. In two animals with intact carotid sinus nerve, no BDA labeling was observed within NTS. In addition, no BDA labeling was observed in the two animals with transection of the ipsilateral carotid sinus nerve. Similarly, no labeling in NTS was observed following an injection into the petrosal/nodose ganglia complex and transection of the vagus and glossopharyngeal nerves. Finally, direct application of the BDA solution to the carotid sinus region did not result in labeling of afferent fibers within NTS. However, in all cases following microiontophoretic injections into the petrosal/nodose ganglia complex, BDA labeled fibers were found extensively within the NTS region as previously described using tranganglionic transport of horseradish peroxidase [19].

Microiontophoretic injections of BDA into the carotid body resulted in anterograde labeling of axons within the ipsilateral caudal NTS. Although few in number these afferent axons within NTS

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