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Original Article

Distribution of leptin receptors in the brain stem: possible route in the pathophysiology of neuromuscular control of airway resistance during sleep

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ABSTRACT

Introduction: Leptin, a hormone related to satiety, has been studied because of its association with obesity and sleep apnea. The distribution of leptin receptors in the brain stem, and in the hypoglossal nucleus, has not yet been described. The stimulation of these muscles has been studied in the treatment of sleep apnea.
Objective: to detail the presence of leptin receptors in the nuclei of these nerves to enable studies of stimulation of this region through leptin. Methods: the brains of five cadavers, removed during necropsy, collected at the Death Verification Service were included. An informed consent was signed by a family member (wife, mother or son/daughter) who answered specific questionnaire concerning comorbities. Anthropometric measurements were recorded. The medulla oblongata and pons fragments were identified. Immunohistochemical staining analysis was performed to identify the location of the leptin receptors.
Results: In the immunohistochemical analysis an intense staining signal of the brownish coloration of neurons was evidenced in the hypoglossal nerve nucleus, moderate in the olivary nucleus and mild in the dorsal nucleus of the vagus and trigeminal nucleus. In motor neurons, more intense brown pigmentation can be observed in the nucleus and cytoplasm when compared to sensory neurons.
Conclusion: The immunoexpression of leptin receptor was demonstrated in the motor neurons of the human hypoglossal nucleus. These results may contribute to unravel details of the pathophysiology of neuromuscular control of airway collapse during sleep and to the development of new drugs capable of improving the neuromuscular tone of upper airway in apneic individuals.

1. Introduction

Leptin is a hormone related to satiety and food intake control. It has been widely studied in recent decades because of its association with obesity, diabetes, and respiratory disorders. This hormone comprises 167 amino acids and is encoded by the *OB* gene, on the long arm of chromosome seven. It is found in a high level in the adipose tissue as it is produced by adipocytes. Its expression in the gastrointestinal tract has also been reported [1–3].

The circulation of leptin in the bloodstream occurs either in free form or bound to the sLEPR protein. Both forms of leptin are metabolized in the kidneys. Free leptin is the bioactive form, and its level increases in obese individuals [2]. Leptin enters the central nervous system through the LEP-Ra receptors, a short isoform of the leptin receptor (LEP-R) present in the blood–brain barrier endothelium. This transport through the blood–brain barrier is clearly decreased in obese rodents and can be considered inversely proportional to serum triglyceride levels. In addition to the leptin receptors of the blood–brain barrier, tanycytes (ependymal cells of the third and fourth ventricles) can also carry leptin to the neurons of the hypothalamus [4–10].

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Leptin receptors (LEP-R or OB-R) are encoded by the *DB* gene and belong to the type I cytokine receptor family [2]. These receptors have an extracellular part comprising 840 amino acids, a transmembrane part comprising 34 amino acids, and an intracellular part of a variable size depending on the isoform [9–13].

The long-isoform OB-RB receptor contains binding sites for signaling proteins, namely, Janus tyrosine kinase two and STAT3. These binding sites are not present in short isoforms [2,10,11]. OB-RB receptors are expressed in rodents and human brain, anterior and posterior hypothalamus, choroid plexus, and cerebellum as well as in other peripheral tissues [2,10,13].

The effects of leptin on weight reduction may be mediated by the activation of JAK–STAT signal transduction in the intracellular OB-RB domain in the hypothalamus [11,13]. Leptin also activates the insulin receptor phosphatidylinositol-3-OH kinase, signaling the hypothalamus to reduce food intake and increase energy expenditure [14,15]. Berger et al. described the activation of the hypoglossal nucleus by the administration of leptin in mice, with consequent stimulation of the pharyngeal and hypopharyngeal muscles, improving obstructive sleep apnea (OSA) indices. This important finding considered as another possible function for leptin: to stimulate the hypoglossal nucleus, maintaining the tone of the oropharynx musculature, preventing its collapse [16].

The existence of this hormone was initially suggested by Coleman (1969) while studying mice with the *db/db* obesity mutation. These mice had obesity, hyperphagia, and diabetes [1]. Coleman performed a parabiosis experiment between *db/db* obese mice and control (nonobese) mice, surgically joining two mice by skin-to-skin anastomosis from the shoulder to the pelvic girdle and vessel anastomosis, establishing a cross-circulation between them. After joining the mice, the nonobese mice died within a short period. Necropsy revealed no glycogen in the liver and empty stomach and intestines. These results suggested that *db/db* obese mice produced such a powerful satiety factor that when carried in the blood, it could induce the normal partner to starve, even with limited cross-circulation between the parabiotic pair members [1]. This substance was later identified in 1994 by Jeffrey Friedman and called leptin [2,3].

Additional studies involving mutant *db/db* mice found that this hormone was encoded by the *OB* gene, also called *LEP* gene, was produced in the adipose tissue, and had receptors in the ventromedial and arcuate nuclei of the hypothalamus, providing negative feedback to the control of fat mass through appetite modulation [1].

Mutations in the leptin gene in mice and humans are the starting point for studies that investigate the molecular and cellular pathways involved in obesity, a metabolic disease with physiological and molecular bases still unclear [2].

A hypothesis that arises then is that the administration of exogenous leptin could resolve obesity issues in mutant mice. However, previous studies have suggested that intravenous or oral leptin therapy are ineffective in curing obesity because of the resistance of the involved receptors to leptin, which does not allow it to pass through the blood–brain barrier, mainly the short isoforms, like OB-Ra, that are involved in the endocytosis and transport of leptin through the blood–brain barrier [1,2,9–13,17]. A leptin-resistant state seems to be an inevitable result of chronic leptin signaling and causes a cycle that is extremely difficult to interrupt [2].

Recent studies in mice have shown that when leptin is intranasally administered, it acts on the central nervous system, decreasing food intake and, consequently, body weight. An improvement in obstructive sleep apnea (OSA) has been reported to occur with the administration of intranasal leptin by increasing the tone of the oropharynx musculature, preventing its collapse [16]. In this study, mice with diet-induced obesity with a high fat diet for 16 weeks, received intranasal leptin (single dose of 0.4 mg/kg). The authors observed that acute intranasal leptin administration decreased oxygen desaturation events in REM sleep, and increased ventilation in NREM and REM sleep. Chronic intranasal leptin administration decreased food intake and body weight. However, the intraperitoneal administration had no effect. The connection pathways of leptin to the hypothalamic and medullary centers are demonstrated by the phosphorylation of the STAT3 protein in areas that express the OB-RB leptin receptor. STAT3 phosphorylation was proved positive in the hypoglossal and vagal nuclei after the intranasal administration of leptin [16].

OSA is closely associated with obesity, because of an increased neck circumference, cardiovascular and metabolic complications, increasing cardiovascular morbidity, and mortality. OSA is characterized by recurrent collapse of the upper airway during sleep resulting in snoring, hypercapnia, hypopnea and apnea. The main treatment for OSA is continuous positive airway pressure (CPAP), but there is a low adherence by patients, leading to an endless search for new treatments [16–20]. Undoubtedly, weight loss is an important key to the treatment of apnea, but this goal is not always easy to achieve. Weight loss improves lung function, decreases neck and abdominal circumference measurements and decreases the risk of metabolic, osteoarticular and cardiovascular diseases. Several authors emphasize improvement in these parameters as well as in sleep ventilatory disorders in obese patients after bariatric surgery. Turnbull et al., in an MRI study in obese patients associated increased volume of upper airway structures with worse OSA parameters. Considering the important role of leptin in satiety and, consequently, in weight loss, as well as its possible stimulation of the hypoglossal nucleus, increasing the tone of the oropharynx muscles and preventing collapse, it encourages the use of leptin in the treatment of sleep disorders [16,21,22].

Some authors have studied in animals the relationship between leptin and OSA by analyzing its role in the central regulation of upper airway permeability and diaphragmatic control. Leptin deficiency and leptin resistance in mice are associated with a higher occurrence of flow limited breathing and pharyngeal obstruction. Expression of leptin receptor ObRb in the hypoglossal nucleus indicates a possible direct role of leptin in OSA [23,24]. Respiratory changes presented by obese mice can be minimized with the administration of leptin [23]. Leptin can also modulate genioglossal muscle activity reducing the collapse of the airways during sleep [24].

Yao et al. [24] studied the effects of intracerebrovascular leptin administration on upper airway obstruction and respiratory control during sleep in mice (ob/ob). The minute ventilation increased after administration. Inspiratory flow limitation and obstructive hypopneas were attenuated by leptin administration to the lateral but not to the fourth cerebral ventricle. They concluded that relieves upper airway obstruction in sleep apnea by activating the forebrain, possibly in the dorsomedial hypothalamus. In contrast, leptin upregulates ventilatory control through hindbrain sites of action, possibly in the nucleus of the solitary tract.

Another treatment proposal for OSA also seek to stimulate the oropharyngeal musculature to prevent its collapse, such as electrostimulators implants. Studies have shown a significant improvement in apnea and hypopnea indices in patients with hypoglossal nerve electrical stimulator implants, even in the long term [19,20,24–26]. This alternative treatment with stimulator implants can generate discomfort, and it highlights the importance of researching other possible ways to stimulate the hypoglossal nerve.

The hypoglossal efferent nerve portion innervates tongue extrinsic muscles such as genioglossus, hyoglossus, styloglossus, geniohyoid and thyrohyoid muscles, responsible for the tongue

movement, and also innervates the intrinsic muscles, as the superior and inferior longitudinal, transversal and vertical muscles, responsible for the tongue structure maintenance. These muscles ensure the elaborate performance of articulation, swallowing, mastication and speech, in addition to maintaining the tongue in an anatomical position, without dopping the base of the tongue, which is associated with airway obstruction [27]. Tongue position and its tone could be guaranteed with hypoglossal nucleus stimulation preventing sleep apnea [16].

As studies in rodents suggest the possible retrograde stimulation of the hypoglossal nucleus through leptin improving respiratory parameters in OSA, this motivated us to study its receptors in the central motor nuclei in humans. The distribution of leptin receptors has already been described in many tissues of the human body, such as the liver, lung, ovary, and hypothalamus. However, to our knowledge, the distribution of these receptors in the brain stem, and specifically in motor nuclei such as the hypoglossal nucleus, has not yet been described in detail in humans [2,3]. We did not identify studies in humans related to leptin receptors in the hypoglossal and vagus nuclei, which motivated us to carry out the present study. Human studies, although important, have especially ethical limitations that make it difficult to carry out them. Because the hypoglossal and vagus nerves are responsible for the innervation of the pharyngeal and hypopharyngeal muscles, this study aimed to detail the presence of leptin receptors in the nuclei of these nerves to determine whether the experiments previously performed with rodents are applicable to humans and could be alternatives in the treatment of OSA [16,24].

2. Materials and methods

For this observational study, the human brains from five adult cadavers removed during necropsy, collected at the Death Verification Service of the city of São Paulo, Brazil, were included. The study was previously submitted and approved by the ethics committee of the Hospital das Clínicas, Faculty of Medicine of São Paulo University. This approval is consistent with the protection of patient and family privacy and confidentiality. An informed consent was signed by a family member (wife, mother, siblings, daughter or son) who answered specific questionnaire concerning, comorbidities, habits, and addictions.

This sample was limited to five cases due to the non-inclusion of cases with deaths from neurological causes, such as tumors, vascular alterations, central nervous system malformations, among others that could alter the local anatomy and compromise our analyzes. Also no cases were collected in which family members did not agree to participate in the study.

Anthropometric measurements such as age, sex, weight, height, body mass index, neck circumference were registered as well as other comorbidities (cardiovascular disease, diabetes, hypertension and smoking). The sample size corresponded to the number of cadavers obtained during the study period. No previous sample calculation was performed.

During the autopsy procedure, after the removal of the brain, the brainstem was properly identified and dissected with care to preserve the medulla oblongata and the pons, avoiding lacerations during handling. Cases with post mortem interval above 8 h were excluded from the study.

2.1. Identification of the hypoglossal nucleus location

The five pons fragments were dissected in the dimensions 5 mm × 10 mm × 5 mm and didactically identified with the letter "P"; the six medulla oblongata fragments were dissected with the same dimensions and identified with the letter "B" (Fig. 1).

For greater accuracy in identifying the location, the fragments received a sequential numbering starting from the transition between the pons and the medulla oblongata. Thus, the medulla oblongata fragments were identified as B1, B2, B3, B4, B5, and B6, and the pons fragments were identified as P1, P2, P3, P4, and P5. B1 was the medulla oblongata fragment closest to the pons, and P1 was the pons fragment closest to the medulla oblongata. According to the anatomical atlas and its correlation with hematoxylin–eosin histological slides, the level of the B2 fragment was considered the most appropriate location for identifying the hypoglossal nucleus (Fig. 1), image created with BioRender © [28].

The study was approved by the Human Research Ethics Committee of the University of São Paulo, Brazil, under the protocol number 05219119.8.0000.0065. The anatomical parts were removed during the autopsy session collected at the Death Verification Service of the city of São Paulo, Brazil. The cause of death was confirmed on the death certificate. Medical records were consulted, ensuring confidentiality of patient data. The closest family members (wife, mother, siblings, daughter or son) signed an informed consent form authorizing the removal of the anatomical part for the study. In unauthorized cases, the decision of the family member was respected. During the removal of the anatomical part, surgical dissection techniques were used with delicate tissue manipulation, performed by two professional surgeons and authors of the study.

2.2. Light microscopy

- Sample preparation for hematoxylin–eosin (HE) staining

For the light microscopy study, the fragments were immediately preserved in 10% formalin with subsequent paraffinization of the specimen, from which histological slides were made. For this purpose, 6-μm thick slices were prepared and stained with hematoxylin–eosin (Fig. 2A – B). Hematoxylin–eosin is a simple technique for staining tissues, suitable for the identification and differentiation of basic substances such as cytoplasm and acidic substances such as cellular nuclei, in this way we consider it suitable for histological identification of these structures.

- Sample preparation for immunohistochemical staining

An immunohistochemical analysis was performed using the paraffinized slides from the previous step to identify the location of the leptin receptors by polymer method (Novolink™).

In brief, following this sequence was applied: deparaffinization; antigen recovery, performed with a 10:1 citrate solution (ScyTek, Inc.), pH 6.0, in a Pascal pressure chamber at 125 °C; endogenous peroxidase block using a 1:1 solution of methanol and hydrogen peroxide for 15 min; rinsing in a phosphate buffered saline solution; protein block (Novolink™ Protein Block) for 15 min; overnight incubation with the primary antibody, a polyclonal anti-leptin receptor antibody, ab60042 (Abcam®), at a 1:1600 dilution; incubation with secondary antibody (Novolink™ Post Primary) for 30 min; incubation with polymer (Novolink™ Polymer) for 30 min; development with DAB 1:50 for 5 min; counter-staining of the nucleus with hematoxylin for 30 s; and assembly with Entellan.

For the immunohistochemical analysis, two observers counted the cells observed in the topography of the nucleoli at 400x magnification with light microscopy. The cells were classified as positive or negative for immunohistochemistry. Subsequently, a comparison was made between the averages of the percentages obtained in the hypoglossal nucleus, dorsal nucleus of the vagus, trigeminal nucleus and olivary nucleus (Fig. 3A–D). Areas with more than 80% of stained nuclei in the observed sites were considered as intense positivity, areas with 80%–60% stained nuclei

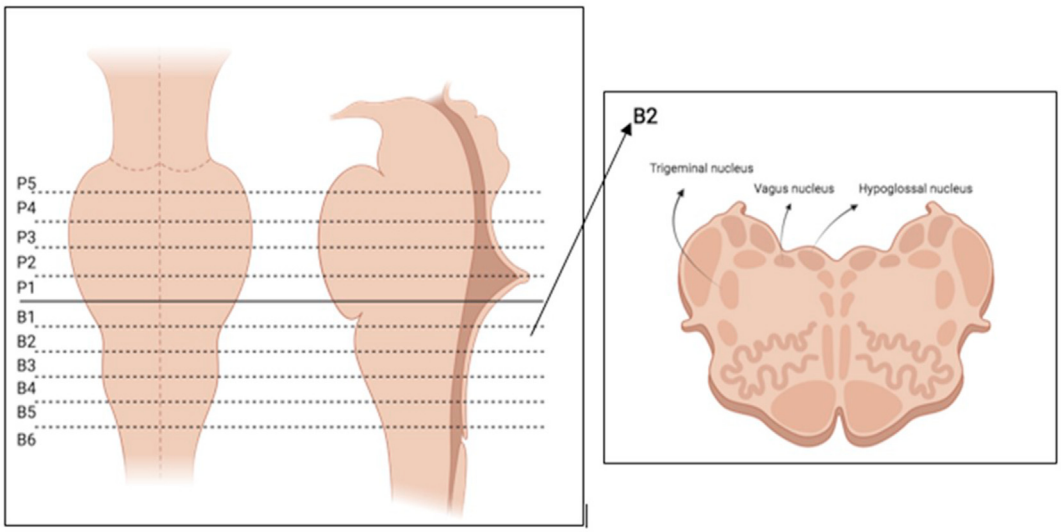

Fig. 1. Brain stem sections.

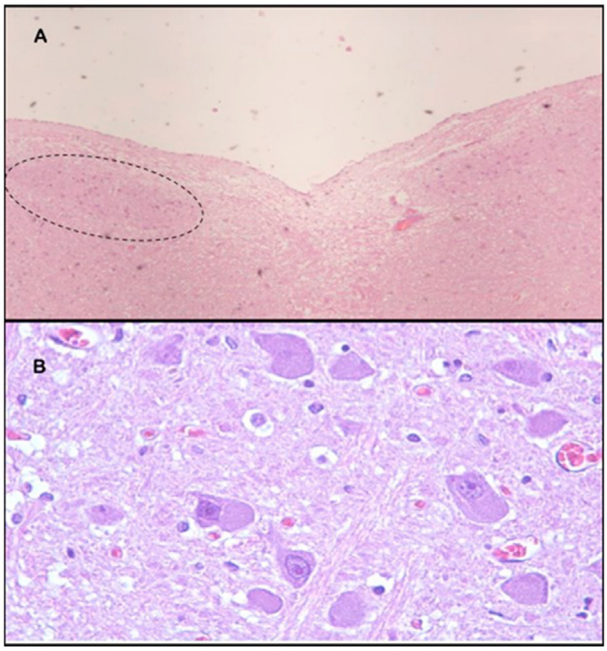

Fig. 2. Light microscopy results of the hematoxylin–eosin staining showing the hypoglossal nucleus. A: 40x magnification, B: 400x magnification.

was considered with moderate positivity and areas with less than 60% of stained nuclei was considered with mild positivity.

The structure of the arcuate nucleus of hypothalamus was used as a positive control (Fig. 3E).

3. Results

- **Anthropometric measurements**

The brain stems of five autopsy cases of adult males aged 31–69 (mean 54.2) years were studied. The body weight ranged from 60 to 109 (mean 85.6) kg; the BMI ranged from 19.8 to 33.9 (mean 27.9) kg/m^2, neck circumference ranged from 37 to 53 (mean 45.02) cm. Smoking history, family history of cardiovascular diseases and causes of death are presented in Table 1 (Table 1).

- Histopathological and immunohistochemical analysis

The nuclei of the vagus, hypoglossal, trigeminal nerves and olivary complex were identified on the HE staining (Fig. 2A–B), and the positivity for leptin receptor on these structures was evaluated in the sequential formalin fixed paraffin embedded (FFPE) preparations (Fig. 3A–C).

In the immunohistochemical analysis an intense staining signal of the brownish coloration of neurons was evidenced in the hypoglossal nerve nucleus (96%), moderate in the olivary nucleus (76%) and mild in the dorsal nucleus of the vagus (40%) and trigeminal nucleus (54%). In motor neurons, more intense brown pigmentation can be observed in the nucleus and cytoplasm when compared to sensory neurons (Fig. 3A–D).

4. Discussion

This study identified more intense immunoexpression of the leptin antibody in the central motor nuclei of the hypoglossal nerve and olivary nucleus, and less intense in the vagus and trigeminal nuclei. Although the sample size of this study was small, the leptin receptor was observed in all cases, indicating the histological presence of this receptor in the studied sites. Other sites in humans where leptin receptors have been identified include lungs, kidneys, adipose tissue, ovaries, testicles, liver, and carotid bulbs. In the central nervous system, the presence of leptin receptor in the choroid plexus and the anterior hypothalamus was described by Burguera et al. [11].

We know the important role of the hypoglossus in the maintenance of lingual tone. Thus, it is assumed that the stimulation of its motor nucleus may result in nerve contraction and, consequently, increase in airway permeability at this anatomical level, especially during sleep, benefiting individuals with sleep-disordered breathing. The action of leptin on the lingual musculature had already been demonstrated by Polotsky et al. when they performed electromyography of the genioglossus muscle in leptin

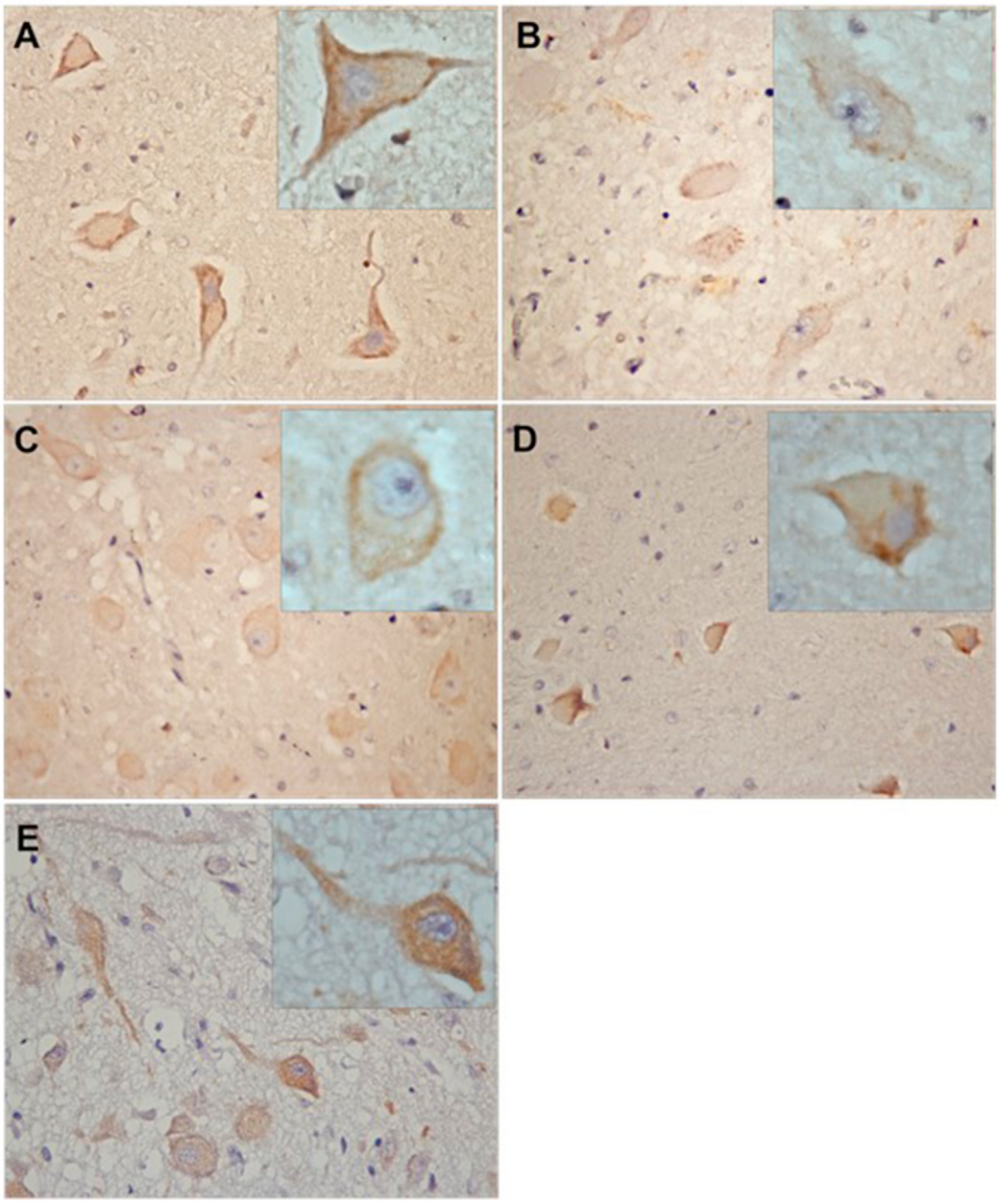

Fig. 3. Light microscopy results showing immunohistochemical positivity in 400x magnification. A: nucleus nervi hypoglossi, B: nucleus dorsalis nervi vagi, C: nucleus spinalis nervi trigemini; D: nucleus olivaris inferior, E: hypothalamus nucleus arcuatus.

Table 1
Anthropometric measurements, habits and comorbidities.

Case number	Case 1	Case 2	Case 3	Case 4	Case 5
Age (year and months)	68y10m	59y 9m	31y 8m	44y 10m	69y 7m
sex	Male	Male	Male	Male	Male
Weight (kg)	85.8	80	109	60	94
Height (m)	1.76	1.73	1.79	1.74	1.72
Body mass index (kg/m^2)	27.7	26.7	33.9	19.8	31.7
Neck circumference (cm)	45.6	38.5	51	37	53
Smoking	Yes	Yes	No	no	Yes
History of Obesity treatment	No	No	No	No	no
Diabetes	Yes	No	No	No	Yes
hypertension	Yes	Yes	No	Yes	yes
Family history of cardiovascular disease	Yes	Yes	Yes	Yes	yes
Cause of death	Acute myocardial infarction	Acute myocardial infarction	bronchopneumonia	Pulmonary thromboembolism	Aortic aneurysm rupture

deficient animal models and observed an increase in the activity of this muscle after subcutaneous administration of leptin, concluding that leptin modulates neuromuscular response to upper airway resistance [29].

Shapiro et al. conducted a study with 23 obese women candidates for bariatric surgery and three men seeking to determine the degree of airway collapse under hypotonic conditions (critical pharyngeal pressure), active neuromuscular responses to upper airway obstruction during sleep, and overnight fasting serum leptin levels. The authors evaluated peak inspiratory airflow (VImax), inspired minute ventilation (VI), and pharyngeal critical pressure (PCRIT) between the active and passive conditions. Increases in serum leptin concentrations were significantly associated with increases in VImax, VI, and PCRIT, independent of BMI, waist-to-hip ratio, neck circumference, or sagittal girth. The authors conclude that leptin may augment neural compensatory mechanisms in response to upper airway obstruction, minimizing upper airway collapse during sleep [30]. However, the authors do not detail the action mechanism of leptin in this neuromuscular control. Perhaps, the immunohistochemical results of our study, detecting leptin receptors in the central motor nuclei of the hypoglossal nerve, may clarify part of the pathophysiology of this neuromuscular control, which contributes to the increase in airway permeability.

The confirmed presence of leptin receptors in the hypoglossal nuclei shows a possibility of retrograde stimulation, as previously reported by Berger et al. in their study involving mice. Their study demonstrated the stimulation of the hypoglossal nucleus through the administration of intranasal leptin, with a consequent contraction of the oropharyngeal muscles and decreased OSA events [16]. In intranasal administration, no influence of a possible resistance of the blood–brain barrier receptors is noted; this might be because leptin reaches the cerebrospinal fluid through perineural and perivascular nasal transport and thus reaches the hypoglossal and vagal nuclei, causing an increased contraction of the genioglossus muscle and the diaphragm [16,20,31].

Freire et al. [32] also discussed the effects of opioids on the upper airway and hypoglossal motor activity and the association of opioid use and obstructive sleep apnea, giving importance to the stimulation of the musculature of the oropharynx through the hypoglossal nucleus for the treatment of sleep apnea.

In a recent study Fleury Curado et al. [33]. activated the genioglossus muscle and improved pharyngeal patency and breathing during sleep in mice through the intralingual administration of designer receptors exclusively activated by designer drugs.

The study of pharyngeal muscle retrostimulation through medications still requires advances, but it has already showed promising results in the treatment of sleep apnea.

Although the study was carried out with a small sample size, we were able to detect the immunosuppression of leptin receptors in the hypoglossal nucleus. Similar studies with a larger sample size should be encouraged to corroborate these findings. We also consider the use of polyclonal antibody a limiting factor in the study, but despite this, the labeling was significantly higher in the hypoglossal nucleus.

5. Conclusions

The immunoexpression of leptin receptor was demonstrated in the motor neurons of the human hypoglossal nucleus. This is the first study in humans with this findings and it corroborates with the observed findings in murine models. Our results may contribute to the development of new drugs capable of improving the neuromuscular tone of upper airway in apneic individuals.

Credit author statement

Maira Garcia Martins: Performed the experiment and data collection; Preparation, creation and laboratorial analyses. Presentation of the published work; Pedro Augusto Magliarelli Filho-Performed the experiment, data collection and laboratorial analyses; Suely Kazue Nagahashi Marie - Provision of study materials and reagents, laboratory support. Data analyses; Luiz Ubirajara Sennes – Conceptualization of the study, design of Methodology, Management and coordination responsibility for the research activity planning and execution.

Conflict of interest

None declared.

The ICMJE Uniform Disclosffigure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2022.03.017.

References

[1] Coleman DL. A historical perspective on leptin. Nat Med 2010 Oct;16(10):1097–9. https://doi.org/10.1038/nm1010-1097. PMID: 20930752.
[2] Zhang Y, Chua Jr S. Leptin function and regulation. Compr Physiol 2017 Dec 12;8(1):351–69. https://doi.org/10.1002/cphy.c160041. PMID: 29357132.
[3] Friedman J. 20 years of leptin: leptin at 20: an overview. J Endocrinol 2014 Oct;223(1):T1–8. https://doi.org/10.1530/JOE-14-0405. Epub 2014 Aug 13. PMID: 25121999.
[4] Saladin R, De Vos P, Guerre-Millo M, et al. Transient increase in obese gene expression after food intake or insulin administration. Nature 1995 Oct 12;377(6549):527–9. https://doi.org/10.1038/377527a0. PMID: 7566150.
[5] Saad MF, Riad-Gabriel MG, Khan A, et al. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. J Clin Endocrinol Metab 1998 Feb;83(2):453–9. https://doi.org/10.1210/jcem.83.2.4532. PMID: 9467557.
[6] Münzberg H, Morrison CD. Structure, production and signaling of leptin. Metabolism 2015 Jan;64(1):13–23. https://doi.org/10.1016/j.metabol.2014.09.010. Epub 2014 Sep 28. PMID: 25305050; PMCID: PMC4267896.
[7] Hileman SM, Pierroz DD, Masuzaki H, et al. Characterizaton of short isoforms of the leptin receptor in rat cerebral microvessels and of brain uptake of leptin in mouse models of obesity. Endocrinology 2002 Mar;143(3):775–83. https://doi.org/10.1210/endo.143.3.8669. PMID: 11861497.
[8] Banks WA, Coon AB, Robinson SM, et al. Triglycerides induce leptin resistance at the blood-brain barrier. Diabetes 2004 May;53(5):1253–60. https://doi.org/10.2337/diabetes.53.5.1253. PMID: 15111494.
[9] Balland E, Dam J, Langlet F, et al. Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. Cell Metabol 2014 Feb 4;19(2):293–301. https://doi.org/10.1016/j.cmet.2013.12.015. PMID: 24506870; PMCID: PMC3936883.
[10] Chen H, Charlat O, Tartaglia LA, et al. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 1996 Feb 9;84(3):491–5. https://doi.org/10.1016/s0092-8674(00)81294-5. PMID: 8608603.
[11] Burguera B, Couce ME, Long J, et al. The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. Neuroendocrinology 2000 Mar;71(3):187–95. https://doi.org/10.1159/000054536. PMID: 10729790.
[12] Porzionato A, Rucinski M, Macchi V, et al. Expression of leptin and leptin receptor isoforms in the rat and human carotid body. Brain Res 2011 Apr 18;1385:56–67. https://doi.org/10.1016/j.brainres.2011.02.028. Epub 2011 Feb 18. PMID: 21334312.
[13] Couce ME, Burguera B, Parisi JE, et al. Localization of leptin receptor in the human brain. Neuroendocrinology 1997 Sep;66(3):145–50. https://doi.org/10.1159/000127232. PMID: 9380271.
[14] Liu J, Yang X, Yu S, et al. The leptin signaling. Adv Exp Med Biol 2018;1090:123–44. https://doi.org/10.1007/978-981-13-1286-1_7. PMID: 30390288.
[15] Yuan X, Caron A, Wu H, et al. Leptin receptor expression in mouse intracranial perivascular cells. Front Neuroanat 2018 Jan 23;12:4. https://doi.org/10.3389/fnana.2018.00004. PMID: 29410615; PMCID: PMC5787097.
[16] Berger S, Pho H, Fleury-Curado T, et al. Intranasal leptin relieves sleep-disordered breathing in mice with diet-induced obesity. Am J Respir Crit Care Med 2019 Mar 15;199(6):773–83. https://doi.org/10.1164/rccm.201805-0879OC. PMID: 30309268; PMCID: PMC6423095.
[17] Frederich RC, Hamann A, Anderson S, et al. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med 1995 Dec;1(12):1311–4. https://doi.org/10.1038/nm1295-1311. PMID: 7489415.

[18] Berger S, Polotsky VY. Leptin and leptin resistance in the pathogenesis of obstructive sleep apnea: a possible link to oxidative stress and cardiovascular complications. Oxid Med Cell Longev 2018 Feb 20;2018:5137947. https://doi.org/10.1155/2018/5137947. PMID: 29675134; PMCID: PMC5841044.
[19] Hong SO, Chen YF, Jung J, et al. Hypoglossal nerve stimulation for treatment of obstructive sleep apnea (OSA): a primer for oral and maxillofacial surgeons. Maxillofac Plast Reconstr Surg 2017 Sep 25;39(1):27. https://doi.org/10.1186/s40902-017-0126-0. PMID: 29018786; PMCID: PMC5610953.
[20] Oliven A, Dotan Y, Golibroda T, et al. Electrical stimulation of the whole hypoglossal nerve in patients with obstructive sleep apnea. Sleep Breath 2020 Dec;24(4):1473–80. https://doi.org/10.1007/s11325-019-02011-1. Epub 2020 Jan 6. PMID: 31907824.
[21] Ming X, Yang M, Chen X. Metabolic bariatric surgery as a treatment for obstructive sleep apnea hypopnea syndrome: review of the literature and potential mechanisms. Surg Obes Relat Dis 2021 Jan;17(1):215–20. https://doi.org/10.1016/j.soard.2020.09.019. Epub 2020 Sep 23. PMID: 33371935.
[22] Turnbull CD, Wang SH, Manuel AR, et al. Relationships between MRI fat distributions and sleep apnea and obesity hypoventilation syndrome in very obese patients. Sleep Breath 2018 Sep;22(3):673–81. https://doi.org/10.1007/s11325-017-1599-x. Epub 2017 Dec 2. PMID: 29197986; PMCID: PMC6133118.
[23] Pho H, Hernandez AB, Arias RS, et al. The effect of leptin replacement on sleep-disordered breathing in the leptin deficient ob/ob mouse. J Appl Physiol 2016;120(1):78–86.
[24] Yao Q, Pho H, Kirkness J, et al. Localizing effects of leptin on upper airway and respiratory control during sleep. Sleep 2016;39(5):1097–106.
[25] Zhu Z, Hofauer B, Wirth M, et al. Long-term changes of stimulation intensities in hypoglossal nerve stimulation. J Clin Sleep Med 2020 Oct 15;16(10):1775–80. https://doi.org/10.5664/jcsm.8320. PMID: 32026804; PMCID: PMC7954005.
[26] Olson MD, Junna MR. Hypoglossal nerve stimulation therapy for the treatment of obstructive sleep apnea. Neurotherapeutics 2021 Jan;18(1):91–9. https://doi.org/10.1007/s13311-021-01012-x. Epub 2021 Feb 8. PMID: 33559036; PMCID: PMC8116425.
[27] Moore Keith L, Dalley Arthur F, Agur Anne MR. Clinically oriented anatomy. 8th ed. Lippincott Williams and Wilkins; 2017.
[28] Machado ABM. Neuroanatomia funcional. 3 ed. São Paulo: Atheneu; 2014.
[29] Polotsky M, Elsayed-Ahmed AS, Pichard L, et al. Effects of leptin and obesity on the upper airway function. J Appl Physiol (1985) 2012 May;112(10):1637–43. https://doi.org/10.1152/japplphysiol.01222.2011. Epub 2012 Feb 16. PMID: 22345430; PMCID: PMC3365404.
[30] Shapiro SD, Chin CH, Kirkness JP, et al. Leptin and the control of pharyngeal patency during sleep in severe obesity. J Appl Physiol (1985) 2014 May 15;116(10):1334–41. https://doi.org/10.1152/japplphysiol.00958.2013. Epub 2014 Feb 20. PMID: 24557793; PMCID: PMC4044396.
[31] Dhuria SV, Hanson LR, Frey 2nd WH. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharmacol Sci 2010 Apr;99(4):1654–73. https://doi.org/10.1002/jps.21924. PMID: 19877171.
[32] Freire C, Sennes LU, Polotsky VY. Opioids and obstructive sleep apnea. J Clin Sleep Med 2021 Oct 22. https://doi.org/10.5664/jcsm.9730. Epub ahead of print. PMID: 34672945.
[33] Fleury Curado T, Pho H, Freire C, et al. Designer receptors exclusively activated by designer drugs approach to treatment of sleep-disordered breathing. Am J Respir Crit Care Med 2021 Jan 1;203(1):102–10. https://doi.org/10.1164/rccm.202002-0321OC. PMID: 32673075; PMCID: PMC7781140.