Physiology & Behavior 153 (2016) 109–114

Contents lists available at ScienceDirect

Physiology & Behavior

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

Hindbrain GLP-1 receptor mediation of cisplatin-induced anorexia and nausea

Bart C. De Jonghe [a,*], Ruby A. Holland [a], Diana R. Olivos [b,c], Laura E. Rupprecht [b,c], Scott E. Kanoski [d], Matthew R. Hayes [b,**]

[a] Department of Biobehavioral Health Sciences, School of Nursing, United States
[b] Translational Neuroscience Program, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, United States
[c] Department of Neuroscience, University of Pittsburgh, United States
[d] Department of Biological Sciences, Human and Evolutionary Biology Section, University of Southern California, United States

HIGHLIGHTS

- Cisplatin chemotherapy activates central GLP-1-producing neurons in the NTS.
- Blockade of hindbrain GLP-1 receptors attenuates cisplatin induced delayed anorexia.
- Blockade of hindbrain GLP-1 receptors attenuates cisplatin induced delayed pica.
- Hindbrain GLP-1 receptor blockade attenuates cisplatin induced body weight loss.

ARTICLE INFO

Article history:
Received 9 September 2015
Received in revised form 9 October 2015
Accepted 28 October 2015
Available online 7 November 2015

Keywords:
Chemotherapy
Nausea
Illness
Vomiting
Emesis
GLP-1

ABSTRACT

While chemotherapy-induced nausea and vomiting are clinically controlled in the acute (<24 h) phase following treatment, the anorexia, nausea, fatigue, and other illness-type behaviors during the delayed phase (>24 h) of chemotherapy are largely uncontrolled. As the hindbrain glucagon-like peptide-1 (GLP-1) system contributes to energy balance and mediates aversive and stressful stimuli, here we examine the hypothesis that hindbrain GLP-1 signaling mediates aspects of chemotherapy-induced nausea and reductions in feeding behavior in rats. Specifically, hindbrain GLP-1 receptor (GLP-1R) blockade, via 4th intracerebroventricular (ICV) exendin-(9-39) injections, attenuates the anorexia, body weight reduction, and pica (nausea-induced ingestion of kaolin clay) elicited by cisplatin chemotherapy during the delayed phase (48 h) of chemotherapy-induced nausea. Additionally, the present data provide evidence that the central GLP-1-producing preproglucagon neurons in the nucleus tractus solitarius (NTS) of the caudal brainstem are activated by cisplatin during the delayed phase of chemotherapy-induced nausea, as cisplatin led to a significant increase in c-Fos immunoreactivity in NTS GLP-1-immunoreactive neurons. These data support a growing body of literature suggesting that the central GLP-1 system may be a potential pharmaceutical target for adjunct anti-emetics used to treat the delayed-phase of nausea and emesis, anorexia, and body weight loss that accompany chemotherapy treatments.

1. Introduction

Chemotherapy is accompanied by severe side effects such as nausea and vomiting [i.e. chemotherapy-induced nausea and vomiting (CINV)], anorexia, and weight loss which diminish quality of life and require effective management. Even with currently prescribed anti-emetic drugs [e.g., serotonin type-3 (5-HT3) antagonists, neurokinin-1 (NK-1) antagonists] that provide effective control of acute and delayed chemotherapy-induced vomiting [1,2], a significant number of patients still exhibit treatment-induced anorexia, nausea, fatigue, and other illness-type behaviors, especially during the delayed phase (>24 h) following treatment [3–5]. Therefore, it is critical to investigate the neurobiological mechanisms mediating chemotherapy-induced nausea and disturbances in feeding behavior during the delayed phase to aid in the development of new anti-emetic targets to control malaise following chemotherapy.

The dorsal vagal complex (DVC), which is comprised of the nucleus tractus solitarius (NTS), the adjacent dorsal motor nucleus of the vagus, and area postrema, is often referred to as the chemoreceptor trigger

* Correspondence to: B.C. De Jonghe, Fagin Hall Office 140, 418 Curie Blvd., Philadelphia, PA 19104, United States.
** Correspondence to: M.R. Hayes, TRL building, Office 2219, 125 South 31st Street, Philadelphia, PA 19104, United States.
E-mail addresses: bartd@nursing.upenn.edu (B.C. De Jonghe), hayesmr@mail.med.upenn.edu (M.R. Hayes).

http://dx.doi.org/10.1016/j.physbeh.2015.10.031
0031-9384/

zone in emesis literature [6]. DVC neural processing modulates descending vagal efferent communication to organs of the alimentary canal, thereby regulating gastric emptying and intestinal motility rates, as well as digestive enzymatic/hormonal secretions. Fluctuations in these physiological processes are thought to contribute greatly to emesis and the feeling of nausea and other illness behaviors [6,7]. However, in addition to processing aversive, stressful, and emetic stimuli [8,9], the DVC is also critically involved in the normal regulation of energy balance [10]. NTS neurons process within-meal gastrointestinal (GI)-derived vagally mediated satiation signals and integrate a multitude of circulating hormones and metabolites relevant to energy balance control. Axonal projections from NTS neurons then communicate monosynaptically and polysynaptically with various hindbrain, midbrain, and forebrain nuclei involved in energy balance regulation (see [10,11] for review). Given that the DVC is a critical neural hub for both nausea and feeding behavior, it is logical that neuropeptide/neurotransmitter systems within the NTS may mediate a portion of the energy balance dysregulation and malaise side effects elicited by chemotherapy.

Glucagon-like peptide-1 (GLP-1) is an incretin hormone synthesized and secreted principally from two locations: the L cells in the distal intestine and preproglucagon (PPG) neurons in the NTS [12], and plays an essential role in the regulation of glycemia and energy balance (see [13,14] for review). Accordingly, multiple GLP-1 receptor (GLP-1R) agonists are now FDA-approved for the treatment of type II diabetes mellitus, and more recently for weight loss [15–17]. While these GLP-1R agonists are administered systemically, it is now well established that GLP-1R ligands penetrate the blood brain barrier and have direct action on GLP-1Rs that are expressed widely throughout the central nervous system (CNS) [18–20]. Of note, activation of a subset of these central GLP-1R-expressing nuclei, in particular those expressed within the NTS, suppress food intake in part by eliciting malaise [21]. To this end, GLP-1-expressing neurons in the caudal NTS stand out as an attractive candidate system that may mediate chemotherapy-induced nausea and energy balance-related effects.

While nausea and emesis have been known side effects of GLP-1R agonists for some time (see [14,21] for review), research has only recently focused on whether blockade of GLP-1R can be used to alleviate illness behaviors elicited by aversive and nauseagenic agents [22,23]. Of potential clinical relevance, recent work from Rudd and colleagues [22] has shown that forebrain intracerebroventricular (ICV) GLP-1R antagonist administration (thus providing widespread forebrain and hindbrain access) partially attenuates the acute emesis elicited by cisplatin therapy in the house musk shrew. However, it remains unclear whether central GLP-1Rs also mediate the anorexia and delayed emetic-like behaviors elicited by chemotherapy and whether the hindbrain GLP-1 system is involved in cisplatin-mediated illness behaviors. It is worth noting that while the acute (<24 h)-phase of CINV (hallmarked by repetitive emetic events) is largely attenuated by 5-HT3R/NK1R antagonists, the delayed phase (>24 h) of CINV (hallmarked by nausea and sporadic emesis) is poorly studied and less controlled by 5-HT3R/NK1R-based drugs [24]. Therefore, using a combination of immunohistochemical and behavioral analyses, the present studies provide support for the hypothesis that endogenous GLP-1R signaling in the DVC is mediating, at least in part, the nausea and the delayed anorexia behaviors elicited by cisplatin chemotherapy.

2. Methods

2.1. Animals and drugs

Adult male Sprague–Dawley rats (Charles River Laboratories; 250–265 at time of purchase), housed individually in hanging metal cages under a 12 h light/12 h dark cycle (lights on 0900 h), had ad libitum access to rodent chow (Purina 5001; St. Louis, MO) and water except where noted. All procedures conformed to and receive approval from the institutional standards of The University of Pennsylvania Animal Care and Use Committee.

The GLP-1R antagonist, exendin-(9-39) (American Peptide Company) was dissolved in sterile artificial cerebrospinal fluid (aCSF) for ICV injections. Cisplatin (Sigma) was dissolved in sterile saline for IP injections at a volume of 3 ml/kg body weight.

2.2. Intracerebroventricular cannula implantation surgeries

Rats were anesthetized using a mixture of ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (0.64 mg/kg) and were placed into a stereotaxic apparatus. Each rat was stereotaxically implanted with a guide cannula (26-gauge; Plastics One, Roanoke, VA) with its tip positioned 2.0 mm above the 4th ventricle (coordinates: on the midline, 2.5 mm anterior to the occipital suture and 5.2 mm ventral to the skull, with injector aimed 7.2 mm from skull) [21,25–27]. Cannulae were attached to the skull with dental acrylic and jeweler's screws. For all surgeries, analgesia was provided (meloxicam, 2 mg/kg). At least five days after surgery, 4th icv injection placement was assessed by measurement of the sympathoadrenal-mediated hyperglycemic response to the cytoglucopenia induced by 5-thio-D-glucose (210 μg) dissolved in aCSF [25,28]. Only data from rats showing at least a twofold increase in blood glucose level in response to this treatment were included in the analyses.

2.3. Immunohistochemical analyses of cisplatin-induced c-fos on NTS GLP-1-immunoreactive neurons

Following a week of daily IP injection habituations, ad libitum fed rats (n = 6/drug treatment) received an IP injection of either saline vehicle (3 ml/kg) or cisplatin (6 mg/kg), 2 h into the light cycle. 48 h following IP injections, all rats were deeply anesthetized and transcardially perfused with 0.1 M PBS, pH 7.4, followed with 4% formalin in 0.1 M PBS. Brains were removed and post-fixed in 10% formalin overnight and then cryoprotected in 20% sucrose in 0.1 M PBS at 4 °C for 3 days. Coronal sections (30 μM) were cut from the hindbrain using a cryostat (Leica 3050S; Leica Corp., Deerfield, IL). Brain sections were stored in 0.1 M PBS at 4 °C until processed. Immunofluorescence in the NTS was quantified in each brain (n = 6/treatment) using 3 representative coronal sections from each animal taken at the level of the obex (−14.8 mm from bregma according to the atlas of Paxinos and Watson [29]). Double immunostaining for GLP-1 and c-Fos was performed on free-floating coronal sections according to modified procedures from our previously published studies [30,31]. Briefly, free-floating coronal sections were washed with 1% sodium borohydride followed by 0.1 M PBS. Sections were incubated on a shaker at room temperature for 1 h with a blocking solution consisting of 5% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in 0.1 M PBS-Tx. Sections were subsequently incubated overnight at room temperature with the following primary antibodies: polyclonal goat anti-c-Fos (1:2000, sc-52G, Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-GLP-1(7-37) (1:2000, T-4363, Bachem, Switzerland). Sections were washed with 0.1 M PBS-Tx and then incubated with respective secondary antibodies: donkey anti-goat Alexa Fluor 594 and donkey anti-rabbit Alexa Fluor 488 (1:500, Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 h. Brain sections were then washed and mounted onto glass slides and coverslipped using Fluorogel (Electron Microscopy Sciences; Hatfield, PA). Using fluorescent microscopy (Nikon 80i; NIS-Elements AR 3.0) at 20× magnification, neurons expressing GLP-1 and c-Fos immunoreactivity were quantified by a separate experimenter blinded to treatment conditions for all coronal sections of the caudal brain stem between −14.8 mm and −14.1 mm from bregma.