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Adaptive upregulation of gastric and hypothalamic ghrelin receptors and increased plasma ghrelin in a model of cancer chemotherapy-induced dyspepsia

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ABSTRACT

Chemotherapy treatment can lead to delayed gastric emptying, early satiety, anorexia, nausea and vomiting, described collectively as the cancer-associated dyspepsia syndrome (CADS). Administration of ghrelin (GHRL), an endogenous orexigenic peptide known to stimulate gastric motility, has been shown to reduce the symptoms of CADS induced in relevant animal models with the potent chemotherapeutic agent, cisplatin. We examined the effects in the rat of cisplatin (6 mg/kg i.p.) treatment on the expression of GHRL and ghrelin receptor (GHSR) mRNAs in the hypothalamus and the stomach at a time-point (2 days) when the effects of cisplatin are pronounced. In addition, plasma levels of GHRL (acylated and total including des-acyl GHRL) were measured and the effect on these levels of treatment with the synthetic glucocorticoid dexamethasone (2 mg/kg s.c. bd.) was investigated. Cisplatin increased GHSR mRNA expression in the stomach (67%) and hypothalamus (52%) but not GHRL mRNA expression and increased the percentage of acylated GHRL (7.03 ± 1.35% vs. 11.38 ± 2.40%) in the plasma. Dexamethasone reduced the plasma level of acylated GHRL and the percentage of acylated GHRL to values below those in animals treated with saline alone (7.03 ± 1.35% vs. 2.60 ± 0.49%). Our findings support the hypothesis that an adaptive upregulation of the ghrelin receptor may occur during cancer chemotherapy-associated dyspepsia. This may have a role in defensive responses to toxic challenges to the gut. In addition, our results provide preliminary evidence for glucocorticoid modulation of plasma ghrelin levels.

1. Introduction

Ghrelin (GHRL) is an endogenous orexigenic, anti-emetic and gastrointestinal (GI) prokinetic peptide secreted mainly from the stomach but also from elsewhere, to act on ghrelin receptors (GHSR) within the hypothalamus (particularly the arcuate nucleus) and on gastrointestinal tract vagal, enteric (e.g. [1–5]) and spinal [6] nerve pathways. Patients with cancer being treated with cytotoxic drugs such as cisplatin, may experience a number of undesirable symptoms including emesis, dyspepsia, and anorexia [7]. Administration of exogenous ghrelin has been shown to have the potential to reduce each of these symptoms in relevant animal models treated with cisplatin as an exemplar cytotoxic agent: emesis in the ferret [5]; anorexia in the rat and mouse [8]; delayed gastric emptying in the mouse [8]. In addition ghrelin has been shown to have gastro-protective effects in a rodent model of ischaemia reperfusion injury [9]. Studies in cancer patients with impaired appetites have shown that ghrelin administration can increase energy intake and meal appreciation of a buffet meal [10]. Together, these results reveal the therapeutic potential of ghrelin receptor agonists but in addition, suggest that if released in response to cytotoxic drugs, ghrelin could act as an endogenous agent attempting to ameliorate the undesirable effects of cytotoxic drugs on emesis, food intake and gastric function. To investigate this hypothesis we have examined the effects in the rat of cisplatin treatment on the expression of GHR and ghrelin-R mRNA in the hypothalamus and the stomach, measured 2 days after treatment and at the time when food intake is at a nadir, delayed gastric emptying is established and pica (argued to be indicative of nausea and/or vomiting in rats which lack a vomiting reflex [11]) is present. Expression levels were also measured 7 days after cisplatin when previous studies have shown that the above effects have subsided [11,12]. In addition, plasma levels of ghrelin (acylated and total including des-acyl ghrelin) were measured and the effects on these levels of treatment with the synthetic glucocorticoid dexamethasone, were investigated. The latter has been shown in related studies to reduce cisplatin-induced pica, delayed gastric emptying, reduced locomotor activity and food intake [13] measured over 2 days after cisplatin administration. Our findings support the hypothesis that an adaptive upregulation of the function of ghrelin may occur during cancer chemotherapy-associated dyspepsia. In addition, the results provide preliminary evidence for glucocorticoid modulation of ghrelin levels in the plasma.

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2. Methods

2.1. Animals

Male Wistar rats (350 g to 400 g.) were obtained from A. Tuck & Son Ltd (Essex, UK) and housed in a temperature and humidity controlled room with 12 h light: 12 h dark cycle (lights on at 07.00 am). The rats were caged individually in clear plastic cages with food (Bantin and Kingman, Hull, UK) and water provided *ad libitum*. Body weight, food and water were monitored daily and the overall health of the animals assessed twice daily against pre-set humane end points for the entire experimental period. All experiments were performed under the UK Animals (Scientific Procedures) Act 1986.

2.2. Drug administration

2.2.1. Study 1

Following a 3 day habituation period in the cages, the rats were given an intra-peritoneal injection (i.p.) of either sterile saline (154 mM NaCl, dose volume: 0.4 ml/100 g) or cisplatin (6 mg/kg). The dose of cisplatin used in the current experiments is comparable to that in previous studies, e.g. Refs. [12,14]. The cisplatin injection was prepared at a concentration of 1.5 mg/ml by dissolving 7.5 mg *cis*-platinum (II) diammine dichloride (Sigma, Poole, UK) in 5 ml sterile saline (154 mM NaCl) with 90 s of sonication. Animals given either cisplatin or saline alone were culled either 2 or 7 days following administration and tissues removed for the molecular studies described in Section 2.3 below.

2.2.2. Study 2

An additional study was performed on groups of animals which received either cisplatin (6 mg/kg i.p) or saline (i.p.) as described above on day 1 and in addition received either dexamethasone (2 mg/kg) or saline subcutaneously (s.c.) 1 h before either the cisplatin (6 mg/kg i.p.) or saline (i.p.). This dose is twice that used in the rat by Rudd et al. [14] but in their study the dose of cisplatin used was half that in the present study (3 mg/kg vs 6 mg/kg). In addition, Rudd et al. [14] used the i.p. route for dexamethasone as opposed to the s.c route in the present study which was considered to be more appropriate for minimising peritoneal irritation when 4 injections were required. At 17.00h, depending upon the group, animals received either a further injection of saline (s.c.) or dexamethasone (2 mg/kg s.c.). On day 2 these animals were given either saline (s.c.) or dexamethasone (2 mg/kg s.c.) at 09.00h and 17.00h. Animals were culled at 10.00h on day 3 (i.e. 48 h after the start of the study) without either any further dexamethasone or saline.

2.3. Measurement of expression levels of mRNA

At either 2 or 7 days after either cisplatin or saline treatment, animals in study 1 were killed between 10.00 and 11.00h by a rising concentration of CO_2 and cervical dislocation (Schedule 1 method) and placed on a bed of dry ice for tissue removal. Hypothalami were taken and snap-frozen in liquid nitrogen prior to storage at −70 °C. The stomach was removed from the same animals, opened, contents removed and weighed (see below) and the tissue washed in chilled sterile saline to remove adherent particulate matter. The stomach was then divided into proximal (the non-glandular region with stratified squamous epithelium externally identifiable as the pale region with a relatively thin wall) and distal (glandular region consisting of the "proper" gastric region and the pyloric gland region but excluding the pyloric sphincter and recognisable by the relatively thickened mucosa [15]) by cutting along the demarcating mucosal ridge and then snap-frozen in liquid nitrogen prior to storage at −70 °C. Total RNA was isolated from the hypothalamus using the RNeasy® Mini kit (Qiagen, Crawley, UK) according to the manufacturer's instructions and from the stomach samples using TRIzol® reagent (Invitrogen, Paisley, UK) as previously described [17]. Relative quantification of mRNA transcripts was performed using the RT-PCR-based 5′ nuclease assay with TaqMan® probes (TaqMan assay, reviewed in [16]). Reverse transcriptase (RT) and negative control RT-minus (NoRT) reactions containing random 9-mers and DNase-treated total RNA (0.21 μg for the hypothalamus samples and 1 μg for the stomach region samples) were performed with no template controls (NoRNA) as before [18]. TaqMan assay oligonucleotide primers (Proligo France SAS, Paris, France) and probes (Applied Biosystems, Warrington, UK) were designed and TaqMan analysis performed as previously described [17] (see Table 1 for oligonucleotide sequences). For each transcript measurement, equal amounts of cDNA were added to each reaction (equivalent to 10 or 20 ng total RNA for the mRNAs and 100 pg for 18S

Table 1
Oligonucleotide sequences of gene-specific TaqMan assay primers and probes

Rat RNA (RGD gene symbol)	Accession number (amplicon position in parentheses)	Primer or probe	Primer or probe sequence
Ghrl	AB029433	Forward (sense)	5′-TCCAAGAAGCCACCAGCTAAAC-3′
	(151–276)	Reverse (antisense)	5′-AACATCGAAGGGAGCATTGAAC-3′
		Fluorogenic probe	FAM-5′-CTTCTGCTTGTCCTCTGTCCTCTGGGTG-3′-TAMRA
Ghsr	AB001982	Forward (sense)	5′-CTCCGGGACCAGAACCACA-3′
	(751–880)	Reverse (antisense)	5′-CCAGAGAGCCAGGCTCGAA-3′
		Fluorogenic probe	FAM-5′-CAAACACCACCACAGCAAGCATCTTCACT-3′-TAMRA
Actb	AF122902.1	Forward (sense)	5′-ACCCTAAGGCCAACCGTGAA-3′
	(131–216)	Reverse (antisense)	5′-CACAGCCTGGATGGCTACGT-3′
		Fluorogenic probe	FAM-5′-CCCAGATCATGTTTGAGACCTTCAACACCC-3′-TAMRA
Hprt	M63983	Forward (sense)	5′-GGTGAAAAGGACCTCTCGAAGTG-3′
	(590–683)	Reverse (antisense)	5′-ATAGTCAAGGGCATATCCAACAACA-3′
		Fluorogenic probe	FAM-5′-CCAGACTTTGTTGGATTTGAAATTCCAGACAA-3′-TAMRA
Ppia	M19533	Forward (sense)	5′-ATGAGAACTTCATCCTGAAGCATACA-3′
	(296–401)	Reverse (antisense)	5′-TCAGTCTTGGCAGTGCAGATAAA-3′
		Fluorogenic probe	FAM-5′-CCTGGCATCTTGTCCATGGCAAATG-3′-TAMRA
Rnr1	M11188	Forward (sense)	5′-ACCTGGTTGATCCTGCCAGTAG-3′
	(12–107)	Reverse (antisense)	5′-AGCCATTCGCAGTTTCACTGTAC-3′
		Fluorogenic probe	FAM-5′- TCAAAGATTAAGCCATGCATGTCTAAGTACGCAC-3fs-TAMRA

FAM: fluorogenic probe reporter dye 6-carboxyfluorescein; TAMRA: fluorogenic probe quencher dye 6-carboxytetramethylrhodamine; RGD: rat genome database (http://rgd.mcw.edu/); Ghrl: ghrelin; Ghsr: ghrelin receptor or growth hormone secretagogue receptor; Actb: β-actin; Hprt: hypoxanthine phosphoribosyltransferase; Ppia: peptidylprolyl isomerase A (cyclophilin); Rnr1: 18S rRNA.