Peptides 90 (2017) 55–62

Contents lists available at ScienceDirect

Peptides

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

Glucagon-like peptide-1 (GLP-1) increases in plasma and colon tissue prior to estrus and circulating levels change with increasing age in reproductively competent Wistar rats

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ARTICLE INFO

Article history:
Received 27 October 2016
Received in revised form 30 January 2017
Accepted 21 February 2017
Available online 22 February 2017

Keywords:
Appetite
Estrous cycle
Gut peptides
GLP-1
Ovarian cycle
PYY
Satiety

ABSTRACT

There is a well-documented association between cyclic changes to food intake and the changing ovarian hormone levels of the reproductive cycle in female mammals. Limited research on appetite-controlling gastrointestinal peptides has taken place in females, simply because regular reproductive changes in steroid hormones present additional experimental factors to account for. This study focussed directly on the roles that gastrointestinal-secreted peptides may have in these reported, naturally occurring, changes to food intake during the rodent estrous cycle and aimed to determine whether peripheral changes occurred in the anorexigenic (appetite-reducing) hormones peptide-YY (PYY) and glucagon-like peptide-1 (GLP-1) in female Wistar rats (32–44 weeks of age). Total forms of each peptide were measured in matched fed and fasted plasma and descending colon tissue samples for each animal during the dark (feeding) phase. PYY concentrations did not significantly change between defined cycle stages, in either plasma or tissue samples. GLP-1 concentrations in fed plasma and descending colon tissue were significantly increased during proestrus, just prior to a significant reduction in fasted stomach contents at estrus, suggesting increased satiety and reduced food intake at this stage of the cycle. Increased proestrus GLP-1 concentrations could contribute to the reported reduction in food intake during estrus and may also have biological importance in providing the optimal nutritional and metabolic environment for gametes at the potential point of conception. Additional analysis of the findings demonstrated significant interactions of ovarian cycle stage and fed/fasted status with age on GLP-1, but not PYY plasma concentrations. Slightly older females had reduced fed plasma GLP-1 suggesting that a relaxation of regulatory control of this incretin hormone may also take place with increasing age in reproductively competent females.

1. Introduction

Reduced food intake in reproductively cycling females has been associated with high levels of estradiol in the circulation; rats consume less during estrus, following the earlier peak in ovarian estradiol secretion during proestrus [1–3]. Eckel et al. found a significant reduction in food intake at estrus to be accompanied by a reduction in water consumption and also reported a significant decrease in body mass in *ad libitum* fed rats. Another study in ovariectomised (OVX) female rats further demonstrated that cyclic administration of near physiological estradiol levels allowed maintenance of feeding patterns and body mass, both of which were lost in non-treated OVX control rats [4]. Despite previously observed cyclic changes to food intake and body mass during the reproductive cycle, the optimal nutritional and metabolic environment to ensure healthy gametes at the time of ovulation and potential conception, prior to early development events, is not well characterised. Likewise, the possible roles of appetite-regulating gut hormones at this time have not been explored in detail.

Appetite-influencing hormone concentrations in the peripheral circulation, including ghrelin, PYY and GLP-1, are generally suppressed in conditions of obesity and in type II diabetes [5–9]. Gastrointestinal bypass surgery results in reduced food intake and body mass gain, and rapid improvements in glucose homeostasis in people with type II diabetes. It is also associated with

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http://dx.doi.org/10.1016/j.peptides.2017.02.010
0196-9781/

marked changes to gut hormone secretion, with postprandial levels of satiety-inducing gut hormones being significantly increased [10–13]. With the globally increased prevalence of obesity in younger human populations, new drugs and endogenous hormone combinations are being tested as alternatives to risky gastric surgery treatments that are effective in altering metabolic/gut hormone profiles towards weight loss. In this context, it will be important to establish what effects altering endogenous appetite hormone concentrations may have on females of reproductive age. Before such work is performed, however, it is necessary to determine how appetite hormone levels change during the ovarian cycle, which is the aim of the present study.

PYY and GLP-1 are anorexigenic (appetite-inhibiting) gut peptides that are co-secreted predominantly from the population of L cells in the distal colon in response to intake of specific nutrients in food [reviewed by [14,15]], although recent studies using perfused male rat guts *ex vivo* have demonstrated the presence and variable secretion of both peptides from proximal and distal areas of small intestine [16]. PYY and GLP-1 are released in a biphasic manner and plasma levels increase from approximately 15 min after the start of food consumption, proportional to meal size, to signal satiety [14,17]. Both peptides additionally have physical effects on the gastrointestinal tract, such as delaying gastric emptying via an ileal brake mechanism, and decreasing gastric acid and intestinal secretions [18,19]. Additionally, GLP-1 acts as an incretin, signalling between the gut and pancreatic beta cells to increase glucose-dependent insulin secretion [15]. The incretin properties of GLP-1 have been utilised to treat type-II diabetes and to achieve modest body mass reductions [20]. Little is known about the potential influence of the reproductive cycle hormones' interaction with PYY and GLP-1, but it may be anticipated that during estrus, when food intake is lowest, PYY and GLP-1 may contribute to the anorexigenic tone.

The main objectives of this study were to establish if any changes in circulating and tissue concentrations of the anorexigenic hormones PYY and GLP-1 occurred in relation to previously documented natural food intake changes during the rodent estrous cycle. It is the first study to consider co-secreted PYY and GLP-1 concentrations in the same matched fed and fasted plasma and in corresponding (fasted) colon tissue samples in female rats at each stage of the estrous cycle. This work also took into account the nocturnal feeding patterns of rodents, with quantification of hormone concentrations during the dark phase at each estrous cycle stage. Secondary aims included checking for any age effects, as data analysis of total ghrelin fed and fasted concentrations from the same samples found decreased amounts in slightly older females [21].

2. Materials and methods

2.1. Animals

This work was licensed under the Home Office Animals (Scientific Procedures) Act 1986 and had approval from The Open University Ethics Committee; rats were specifically chosen for this study to obtain enough blood for fed and fasted comparison. Nulliparous female Wistar rats (Harlan, UK, n = 43) were housed in groups of four and maintained under a 12 h reverse light cycle (lights off between 11.00 and 23.00) with free access to standard breeding diet and water. All procedures were carried out during the dark phase so that samples were obtained when most physiologically relevant for natural feeding behaviour. Females were kept near a cage of male rats to keep the females cycling normally. Daily estrous monitoring was undertaken at 24-hourly intervals between 11.00 (lights off) and 13.00 to determine cycle stages by vaginal lavage, as described by Becker et al. [22]. Rats were between 32 and 44 weeks of age at the end of the study. Rats were monitored for a minimum of 2 complete cycles (often up to 4 cycles) to obtain sample groups at each stage of the estrous cycle.

2.2. Blood and tissue collection and preparation

Fed blood samples from the tail vein were taken between 13.00 and 15.00, after completion of estrous monitoring, the day before the cycle stage to be studied. Some animals did not progress as predicted with their estrous cycle, so there is a mismatch between the number of animals in the fasted state cycle stages (proestrus n = 12; estrus n = 11; metestrus n = 9, diestrus n = 11) and the numbers obtained for the fed cycle stages the day before (proestrus n = 14; estrus n = 7; metestrus n = 6, diestrus n = 16).

Rats were fasted from 08.00, before the beginning of the dark period (when maximum food consumption occurs), 4–8 h prior to culling. Cage food was removed at 08.00 the day after tail vein fed sample collection, and animals were then sacrificed between 12.00 and 16.00. Fed blood samples were immediately acidified by dilution at 1:10 in buffer (0.1 M ammonium acetate, 0.5 M NaCl, pH 3.6) as recommended for optimal peptide preservation and recovery [23]. Rats were anaesthetised and decapitated, and a fasted blood sample was obtained from trunk blood. All blood was collected into EDTA coated tubes with a protease inhibitor. Stomachs were removed and masses were recorded both before and after opening along the greater curvature and rinsing in PBS, enabling the mass of remaining stomach contents to be calculated. Samples of descending colon were removed and immediately frozen for later peptide extraction and assay.

2.3. Radioimmunoassays

Samples of fed and fasted plasma and fasted colon tissue extract were analysed according to the manufacturer's protocol for total PYY and GLP-1 concentrations using radioimmunoassay kits of the same batch number per peptide (Millipore, UK). All tissue samples were diluted prior to addition to RIAs using distilled water. Fasted plasma samples for GLP-1 measurement were extracted and added to each kit as outlined in the assay protocol. Samples were added to assays based on sample type, not cycle stage. *PYY:* intra-assay%CV: 2.97 ± 1.617 (n = 3), inter-assay%CV: 2.85 ± 3.172 (n = 3). *GLP-1:* intra-assay%CV: 3.34 ±1.279 (n = 3), inter-assay%CV: 6.28 ± 1.551 (n = 3).

2.4. Statistics

Values represent mean ± S.E.M. Statistical analysis was carried out using a one-way ANOVA with a Tukey post-hoc test on normally distributed data. When data were not normally distributed, and could not be transformed (e.g. by log transformation) to normality, a Kruskal-Wallis test was used, with follow-up pairwise comparisons (Mann-Whitney), with Bonferroni correction. A paired-samples *t*-test was used to compare hormones in the fed and the fasted state. Further statistical testing used a univariate general linear model (GLM) with PYY or GLP-1 concentration as the primary dependent variable and fed/fasted status and stage in cycle as independent variables, with age in weeks as a covariate. To evaluate our secondary aims, Pearson correlations were used to determine if either hormone concentrations were correlated in fed and fasted states and with age. All statistical tests were performed using IBM SPSS Statistics 21. $P < 0.05$ was considered statistically significant.