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SHORT COMMUNICATION

Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in obese type 2 diabetic volunteers

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Abstract
Aims/hypothesis Glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are secreted from enteroendocrine L cells in response to numerous stimuli, including bile salts. Both have multiple effects that are potentially useful in treating diabetes and obesity. L cell number and hormone content in the intestine are highest in the rectum in humans. We investigated the effects of intrarectal sodium taurocholate on plasma GLP-1, PYY, insulin and glucose concentrations, and on food intake of a subsequent meal.

Methods Ten obese type 2 diabetic volunteers were each studied on five separate occasions after an overnight fast and oral administration of 100 mg sitagliptin 10 h before the study. They then received an intrarectal infusion of either one of four doses of taurocholate (0.66, 2, 6.66 or 20 mmol, each in 20 ml of vehicle) or vehicle alone (1% carboxymethyl cellulose) single-blind over 1 min. Hormone and glucose measurements were made prior to, and for 1 h following, the infusion. The consumption of a previously selected favourite meal eaten to satiety was measured 75 min after the infusion.
Results Taurocholate dose-dependently increased GLP-1, PYY and insulin, with 20 mmol doses resulting in peak concentrations 7.2-, 4.2- and 2.6-fold higher, respectively, than those achieved with placebo ($p<0.0001$ for each). Plasma glucose decreased by up to 3.8 mmol/l ($p<0.001$). Energy intake was decreased dose-dependently by up to 47% ($p<0.0001$). The ED_{50} values for effects on integrated GLP-1, insulin, PYY, food intake and glucose-lowering responses were 8.1, 10.5, 18.5, 24.2 and 25.1 mmol, respectively.
Conclusions/interpretation Therapies that increase bile salts (or their mimics) in the distal bowel may be valuable in the treatment of type 2 diabetes and obesity.

Keywords Bile salts · Enteroendocrine · GLP-1 · PYY

Abbreviations

DPP4	Dipeptidyl peptidase IV
FXR	Farnesoid X receptor
GLP	Glucagon-like peptide
PYY	Peptide YY
t_{50}	Time to peak plasma concentration

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Introduction

The disease burden and rising incidence of type 2 diabetes, currently 8.4% in Europe, 13.7% in the USA and 25% in the United Arab Emirates (the location of the current study), compels the search for novel therapies.

Peptide hormones secreted from enteroendocrine L cells include glucagon-like peptide 1 (GLP-1), GLP-2, oxyntomodulin and peptide YY (PYY). GLP-1 agonists have proved particularly successful as glucose-lowering agents [1]. Another recent glucose-lowering class, the dipeptidyl peptidase IV (DPP4) inhibitors, likely also enhance GLP-1 agonism by inhibiting degradation of the active form, GLP-1[7–36]NH_2. Currently, no marketed drugs target stimulation of L cell secretion.

Each of the L cell hormones has been considered as a drug target. In addition to GLP-1, PYY is glucose-lowering [2]. GLP-1, PYY and oxyntomodulin [3] are anorectic and associated with weight loss. PYY and GLP-2 are intestinotrophic.

The density of L cells, as reflected by PYY content per unit weight, increases markedly in humans with distal progression along the gut [4], with content in the rectum being ~100 times that in the duodenum.

In isolated gut preparations, GLP-1 and PYY secretion are stimulated by intraluminal perfusion of nutrients, and especially by bile salts [5]. Intracolonic infusions of deoxycholic acid, a constituent of human bile, dose-dependently increased plasma PYY and enteroglucagon (GLP-1) concentrations [6] in patients undergoing colonoscopy.

The objective of the current human physiology study was to determine whether a bile salt, in this case sodium taurocholate, when applied to the rectal lumen, could invoke secretion of L cells to a therapeutically meaningful extent. Because a large component of the effect of such secretion would come from active GLP-1, a DPP4 inhibitor was co-administered to maximise the probability of observing an effect. Because anticipated effects included acute lowering of fasting glucose, volunteers with type 2 diabetes were recruited to maximise the glucose dynamic range. Because type 2 diabetes and the metabolic syndrome are associated with obesity, obese volunteers were recruited.

Methods

Study volunteers Ten male volunteers, mean age 46.3 years (range 41–56), mean BMI 33.0 kg/m^2 (range 30.5–39.1), with type 2 diabetes of less than 5 years' duration treated by diet alone (1/10) and/or metformin (9/10), were recruited. Volunteers were admitted to the study if fasting glucose was <16.7 mmol/l (300 mg/dl), HbA_{1c} was <97 mmol/mol (11%), liver function tests were normal, they had no chronic illness other than diabetes and they had no history of bowel disease, gastrointestinal surgery or any disease that could affect bile salt metabolism, smoking, alcoholism or gastroenteritis in the prior 6 months. More detailed clinical information on the volunteers is provided in electronic supplementary material (ESM) Table 1, and for inclusion and exclusion criteria and study design see the ESM text. All studies were approved by the institutional ethics committee and each volunteer gave informed consent prior to each investigation.

Study design Volunteers withheld any oral medication during the fasting period prior to the study, and during the study. They were studied on five separate occasions 1 week apart. Ten hours before the study, each volunteer took 100 mg sitagliptin, a DDP-4 inhibitor, orally. After an overnight fast, an indwelling catheter (Intracath, Becton Dickinson, Franklin Lakes, NJ, USA) was placed in a forearm vein for blood sampling and kept patent with saline between 5 ml samples taken into ice-cold EDTA Vacutainers (Becton Dickinson) together with 50 μl of a DPP4 inhibitor (catalogue ref. DPP4-010, Millipore, St Charles, MO, USA). Venous samples were taken 15 and 0 min before, and 10, 20, 30, 40, 50 and 60 min after rectal taurocholate. Cooled samples were immediately centrifuged, and plasma frozen and stored at −80°C. At t=0 min, placebo or sodium taurocholate at doses of 0.66, 2.0, 6.66 and 20.0 mmol (0.36, 1.08, 3.58, 10.75 g) was delivered rectally via a silastic cannula in 20 ml of 1% (wt/vol.) carboxymethyl cellulose over a period of 1 min.

Taurocholate was administered in a single-blind dose-rising design. Side effects were recorded at the end of each session.

At 75 min, a previously chosen favourite meal was presented in excess, and the volunteer asked to eat to satiety. Weight and energy content of food consumed after 2 h was determined by weighing.

Hormone analysis Active human GLP-1 (GLP-1 [7-36] amide), total human PYY and insulin were measured by enzyme-linked immunoassays using Linco assay kits (EGLP-35K, EZHPYYT-66K and EZHI-14K, respectively, Millipore, Billerica, MA, USA). Glucose was measured by the glucose oxidase method using assay kits (item no. 10009582, Cayman Chemicals, Ann Arbor, MI, USA).

Data analysis The effects of taurocholate dose on PYY, GLP1, insulin and blood glucose, adjusted for baseline, were analysed using the mixed model ANOVA (MIXED). The impact of taurocholate dose on food intake was analysed using repeated measures ANOVA. Dose–response and concentration–response analyses used constrained four-parameter fits (Prism v5.04; GraphPad Software, San Diego, CA, USA).

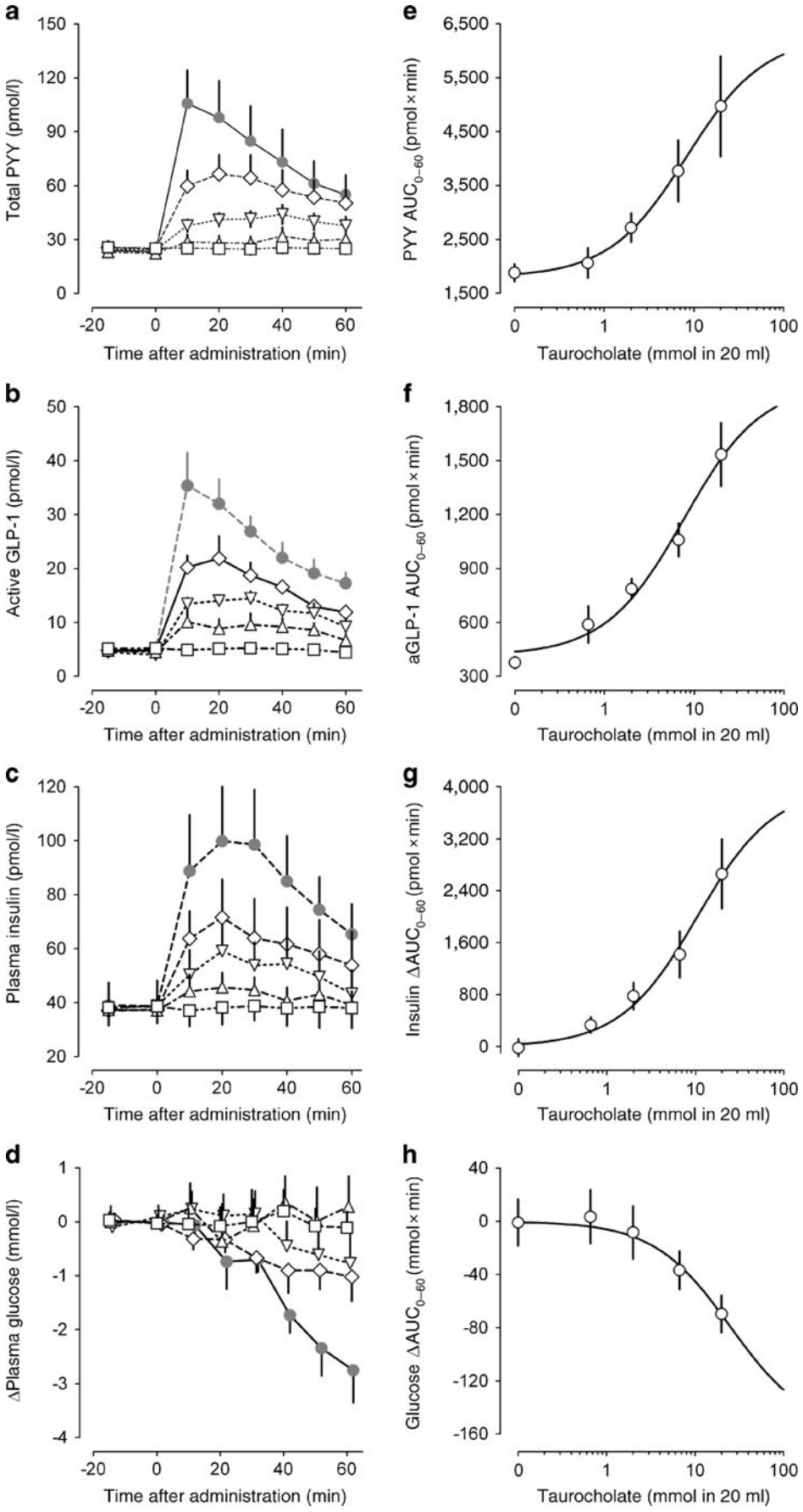

Fig. 1 Time course of effects of rectal administration of sodium taurocholate on plasma (**a**) total PYY, (**b**) active GLP-1, (**c**) insulin and (**d**) change in glucose concentrations in obese diabetic male volunteers, each administered placebo and four doses of taurocholate. Values are means ± SEM, $n=10$. Taurocholate doses are represented by closed grey circles, 20 mmol; diamonds, 6.66 mmol; inverted triangles, 2.0 mmol; upright triangles, 0.66 mmol; open squares, placebo. Mean baseline glucose concentration was 11.90± 0.56 mmol/l. Corresponding dose–response relationships for incremental responses integrated over 60 min for (**e**) total PYY, (**f**) active GLP-1 (aGLP-1), (**g**) insulin and (**h**) glucose change, after taurocholate administration

Results

All ten volunteers received all doses. Rectal administration of sodium taurocholate was associated with dose-dependent increases in plasma concentrations of active GLP-1 and total PYY (Fig. 1, $p<0.001$ for each). Peptide concentrations peaked by the first or second time point (10 or 20 min). Rectal taurocholate (20 mmol) elevated active GLP-1 7.2-fold, from 4.8±0.4 pmol/l (placebo, 10 min) to 35.4±6.1 pmol/l with an ED_{50} of 7.6 mmol. At this dose, PYY increased 4.2-fold to 105.7±18.6 pmol/l with an ED_{50} of 8.3 mmol.

Taurocholate administration dose-dependently increased plasma concentrations of insulin 2.6-fold from 38.2±6.4 pmol/l at 20 min (the typical time to peak plasma concentration [t_{max}]) to 99.9±20.7 pmol/l, with an ED_{50} of 10.5 mmol (Fig. 1, $p<0.001$). Plasma glucose concentration decreased progressively over 60 min by up to 3.8 mmol/l with an ED_{50} of 25 mmol (Fig. 1, $p<0.001$).

Rectal administration of taurocholate was associated with a dose-dependent reduction of up to 47% in food intake in the subsequent 2 h (Fig. 2, $p<0.0001$). The MIXED test showed a significant negative association for GLP-1 on food intake (i.e. high values of GLP were associated with low food intake) but not for PYY. Similarly, individual plasma GLP-1 concentration at $t=60$ min (the last measure before the test meal) predicted food intake better than PYY concentration ($r=0.78$–0.81 vs $r=0.26$–0.52). The derived EC_{50} for the effect of endogenous GLP-1 at 60 min on inhibition of food intake was 17.7 pmol/l.

No side effects were noted with placebo or with the two lowest doses of taurocholate. With the 6.66 mmol dose, three of the ten volunteers reported mild rectal irritation, one had two loose stools, one reported some abdominal pain and a headache, and one reported difficulty urinating. With the 20 mmol dose, six of the ten volunteers complained of rectal irritation, three had mild abdominal pain, three reported mild headache, two had constipation that lasted 2–3 days and one had a feeling of having overeaten.

Discussion

Sodium taurocholate, administered rectally in 20 ml gel, invoked rapid (t_{max} ~10 min) and robustly dose-dependent increases in plasma concentrations of the L cell hormones GLP-1 and PYY in obese type 2 diabetic volunteers previously administered a DPP4 inhibitor. These changes were followed (t_{max} ~20 min) by dose-dependent increases in plasma insulin concentration, and then by declines in plasma glucose concentration (still falling at $t=60$ min). Finally, a dose-dependent satiogenic effect was measured 75 min after administration. We previously showed that intracolonic bile salts released L cell hormones in humans [6]; however, the present study additionally shows dose-responsive effects on insulin secretion, blood glucose concentrations and subsequent food intake.

Sitagliptin was administered to the volunteers in an attempt to increase active GLP-1 concentrations and thus enhance the insulinotropic and satiogenic responses. Although this is likely to have augmented effects driven by active GLP-1, it may have diminished effects due to PYY[3-36], a Y2-selective form derived by DPP4-mediated cleavage of the secreted form, PYY[1-36]. Further, as there is feedback inhibition by active GLP-1 of L cell secretion [7], increasing the gain of that feedback inhibition with sitagliptin may have diminished the L cell secretory response that may have otherwise been invoked. Thus, the L cell responses observed here may represent a conservative picture of the responses possible following bile

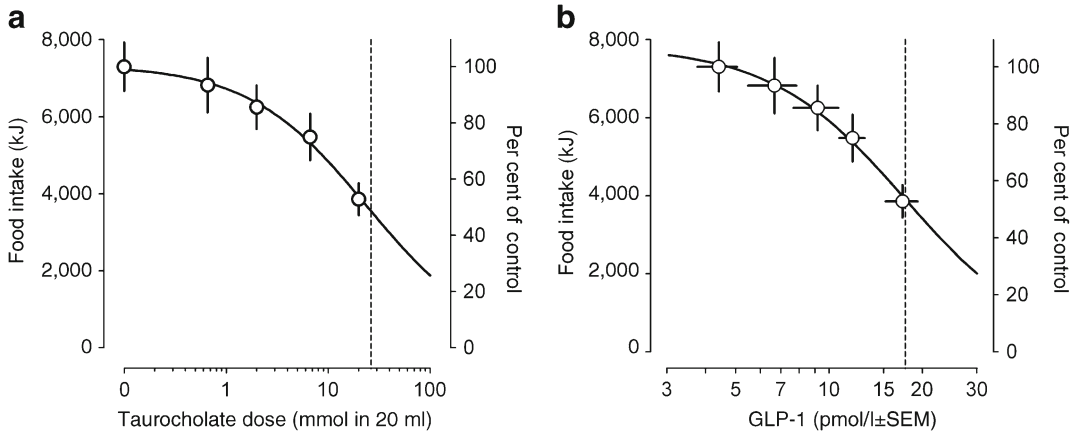

Fig. 2 (**a**) Dose-responsive effect of rectal sodium taurocholate on food intake until satiated in ten obese diabetic volunteers. Values are means ± SEM, $n=10$. Note that the presentation of the meal was 75 min after the taurocholate or the placebo was administered. (**b**) Concentration response for food intake vs the GLP-1 concentrations observed 60 min after taurocholate administration. $EC_{50}=17.6$ pmol/l± 0.07 log. Food intake is expressed as energy consumed (in kJ, left axes) or as a fraction of consumption observed with vehicle alone (right axes). Dotted lines in each panel signify the ED_{50} values of unconstrained fits

salt stimulation. However, because sitagliptin essentially restricts circulating GLP-1 to the active GLP-1[7-36]NH_2 form measured here, our assessment of the L cell response is likely to be valid. This conclusion is supported by the concordance of GLP-1 and PYY responses (R^2=0.96).

The 20 ml dose volume employed here essentially restricted direct contact of taurocholate to the rectum. The abruptness and magnitude of the GLP-1 and PYY secretory responses imply that release into the plasma must have been virtually instantaneous, and suggest mediation via a receptor at the luminal surface. Bile salt receptors TGR5 (also known as G protein-coupled bile acid receptor 1 or GPBAR1) and farnesoid X receptor ([FXR], also known as the bile acid receptor [BAR] or NR1H4) have attracted attention as drug targets for metabolic diseases [8]. The receptors responsible for the effects of taurocholate reported here remain undefined. Selective FXR agonism appears not to be involved in L cell stimulation [9], and in any event would appear too slow in responding to bile salts to accommodate the rapidity of response observed here. And although there is some support for the involvement of TGR5 signalling in L cell stimulation [9], selective and potent non-bile salt TGR5 agonists failed to consistently stimulate L cell secretion (L. Chen, GlaxoSmithKline, Durham, NC, USA, personal communication). Other bile salt-responsive receptors potentially implicated may include one or more of the bitter receptors located on colonic L cells [10].

These findings open up the possibility that intrarectal L cell stimulation with bile salts, or molecules that mimic the cognate pharmacology, whatever that may prove to be, could be useful for the treatment of diabetes and/or obesity. Several approaches to increasing bile salt concentrations in the lower bowel may be possible: rectal administration of bile salts or their agonists, inhibiting the recuperative apical sodium-coupled bile salt transporters in the terminal ileum [11] or otherwise shunting bile salts to the distal large bowel using, for example, a device.

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Duality of interest TEA and BG are members of the Scientific Advisory Board of Satiogen. TEA, BG and AAY hold stock in the same company. The other authors declare that they have no duality of interest associated with this manuscript.

Contribution statement TEA, SG, HS, JAK, BG and AAY contributed to conception and design. TEA, SG, KAP, SAT, NN, BG and AAY contributed to the analysis and interpretation of data. All authors contributed to the drafting and/or critical revision of the manuscript, and gave final approval of the version to be published.

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