# Bile acids stimulate GLP-1 release by accessing basolateral GPBAR1 (TGR5)

Cheryl A Brighton[1], Juraj Rievaj[1], Rune E Kuhre[2], Leslie L Glass[1], Kristina Schoonjans[3], Jens J Holst[2], Fiona M Gribble[1], Frank Reimann[1]

[1] WT-MRC Institute of Metabolic Science, University of Cambridge, UK. [2] NNF Centre for Basic Metabolic Research, University of Copenhagen, Denmark. [3] Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Switzerland.

## Background

Glucagon-like peptide 1 (GLP-1) is an incretin hormone released from enteroendocrine L-cells in the gut. GLP-1 analogues and dipeptidyl-peptidase-4 inhibitors are currently used to treat type-2 diabetes. A greater understanding of the mechanisms underlying the release of GLP-1 may facilitate the development of therapeutics to stimulate the release of endogenous GLP-1. Bile acids have been shown to induce GLP-1 release via the G protein-coupled bile acid receptor 1 (GPBAR1/TGR5) and increased cAMP. The apical sodium-dependent bile acid transporter (ASBT) and nuclear farnesoid X receptor (FXR) may also be involved.

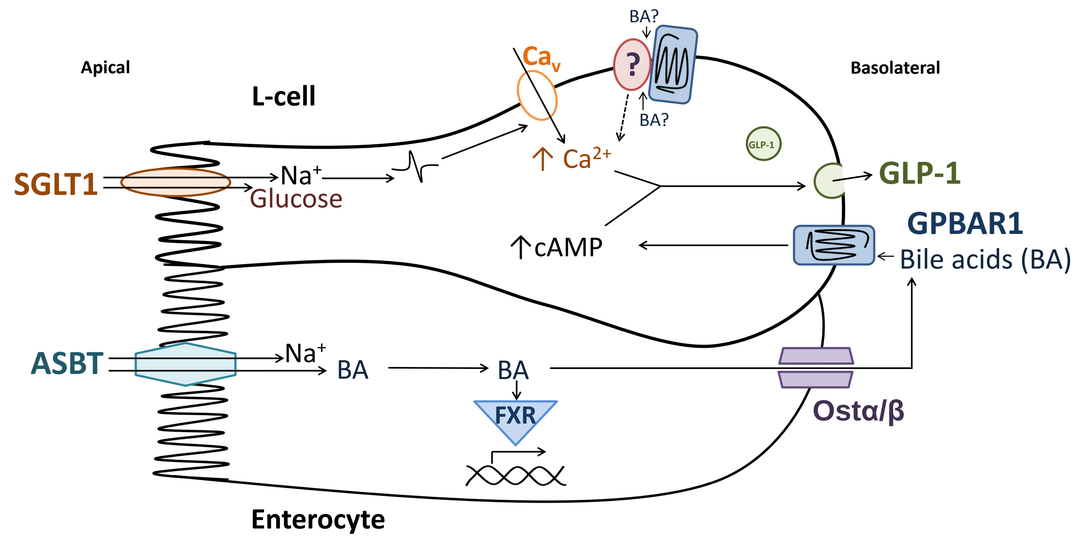
## Approach

Enteroendocrine cells, such as the GLP-1 releasing L-cells, make up less than 1% of intestinal epithelial cells. The study of these specialised cells is facilitated by transgenic mice expressing fluorescent sensors (Epac2camps or GCaMP3) specifically in L-cells. GLP-1 release was measured from primary murine intestinal cultures and tissue segments mounted in Ussing chambers using a MesoScale Discovery assay.

**Aim** To identify pathways of bile acid-stimulated GLP-1 secretion and whether these are activated from the apical or basolateral direction.

## Conclusion

Bile acids stimulate GLP-1 secretion primarily via activation of GPBAR1 on the basolateral surface of intestinal L-cells. This suggests the stimulation of gut hormone secretion may include post-absorptive mechanisms. It could impact the design of therapeutics which target GPBAR1 as a means of increasing endogenous gut hormone secretion.




# Results

## 1. Bile acid-induced GLP-1 secretion

GPBAR1 is activated preferentially by TLCA or its specific agonist GPBAR-A (GP-A), however TDCA is a significantly stronger stimulus at 100µM.

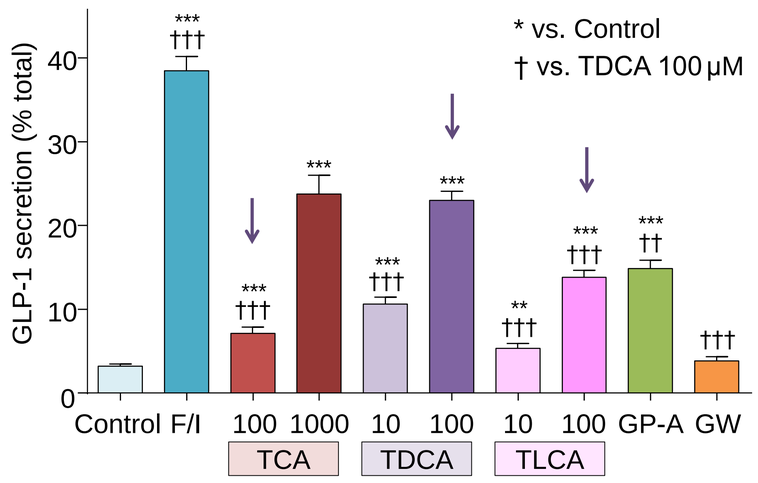



*Figure 1*
***Method:*** *Primary cultures of mouse lower small intestine incubated for 2hr in saline with 10 mM glucose (Control). GLP-1 (total) release is expressed as a percentage of content.*
***Stimuli*** *(µM):, forskolin (10 µM) + IBMX (10 µM) (F/I), bile acids (concentrations as indicated in µM): taurocholic acid (TCA), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA), GPBAR-A (GP-A, 3 µM) and GW4064 (GW, 5 µM).*
***Analysis:*** *Results are means + SEM of 3-5 experiments, each performed in triplicate or quadruplicate (n=12-23). Statistical differences were determined using one way ANOVA and post-hoc Bonferroni on $log_{10}$ transformed data. Statistical difference from basal (*) or TDCA 100 µM (†), **/††p<0.01, ***/†††p<0.001.*

## 2. Intracellular cAMP is increased by bile acids

Measured in intestinal cultures using a FRET-based fluorescent cAMP sensor, Epac2camps, expressed specifically in L-cells.

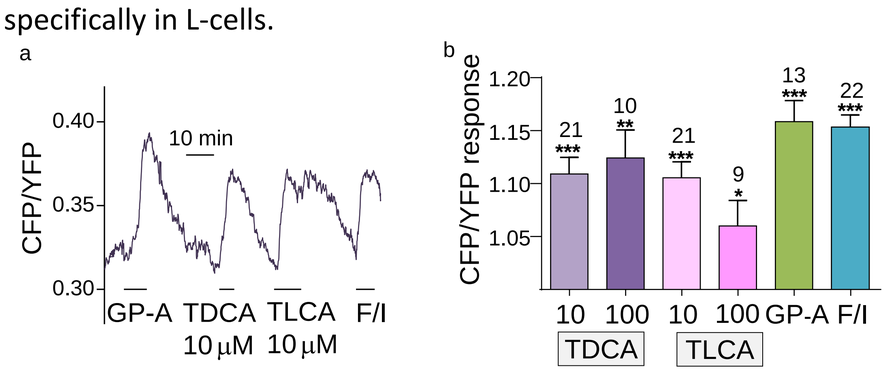



*Figure 2*
***Method:*** *Lower small intestinal cultures were generated from mice expressing Epac2camps in L-cells. Cells were perfused with stimuli as indicated in saline buffer containing 10 mM glucose.*
***Stimuli:*** *GPBAR-A (GP-A, 3 µM), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA) and forskolin (10 µM) + IBMX (100 µM) (F/I).*
***Analysis:*** *Mean fold-change in CFP/YFP ratio, representing [cAMP] (b), for cells recorded as in representative trace (a). Error bars represent SEM, n numbers indicated above bars. Statistical difference from basal was determined by one-sample t-test (* p<0.05, **p<0.01, ***p<0.001).*

## 3. Bile acids stimulate influx of $Ca^{2+}$

Measured in intestinal cultures using a fluorescent calcium sensor, GCaMP3, expressed specifically in L-cells.

- TDCA and TLCA trigger rises in intracellular $Ca^{2+}$.
- Specific agonists of GPBAR1 (GP-A) and FXR (GW) produce minimal calcium responses.
- Use of $CoCl_2$ indicates $Ca^{2+}$ enters via voltage gated calcium channels (not shown).

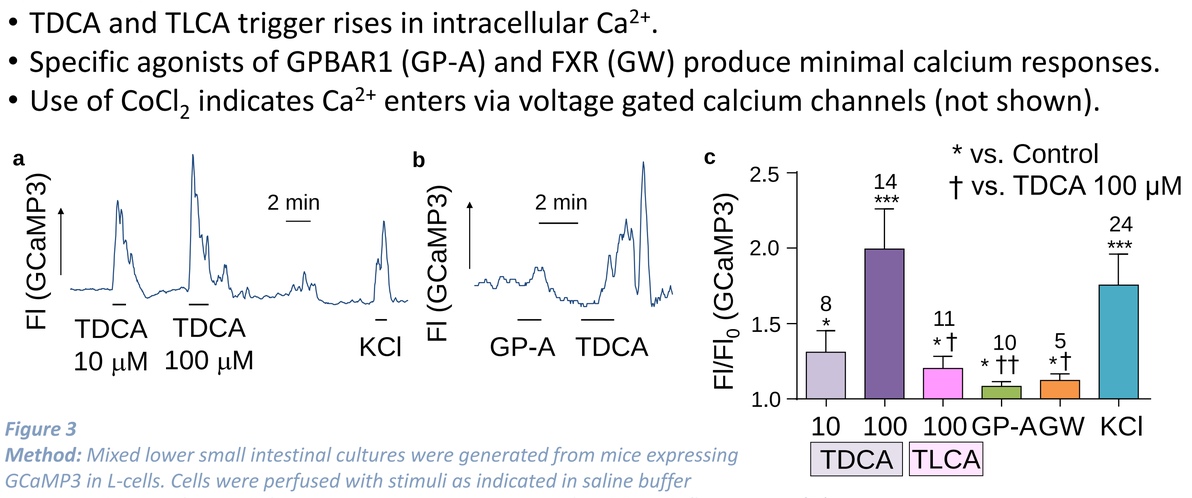



*Figure 3*
***Method:*** *Mixed lower small intestinal cultures were generated from mice expressing GCaMP3 in L-cells. Cells were perfused with stimuli as indicated in saline buffer containing 10 mM glucose. Calcium concentrations are measured as GCaMP3 fluorescence (FI).*
***Stimuli:*** *(a) Taurodeoxycholic acid (TDCA) and 30 mM KCl, (b) GPBAR-A (GP-A, 3 µM) and TDCA (100 µM). GW4064 (GW, 5 µM).*
***Analysis:*** *(c) Mean increase in GCaMP3 fluorescence over baseline ($FI/FI_0$) for cells recorded as in a-b. Cells which did not respond to any stimuli were excluded from analysis (4/28). Results are means + SEM and n numbers are indicated above bars. Statistical difference from basal signal (*) was determined via one-sample t-test and statistical difference from TDCA (†) was determined by one way ANOVA and post-hoc Bonferroni test on $log_{10}$ transformed data (*/† p<0.05, ††p<0.01, ***p<0.001).*

## 4. Bile acid uptake via ASBT

An ASBT-inhibitor (ASBT-I) has no effect upon bile acid induced GLP-1 secretion and intracellular $Ca^{2+}$ changes in intestinal cultures.

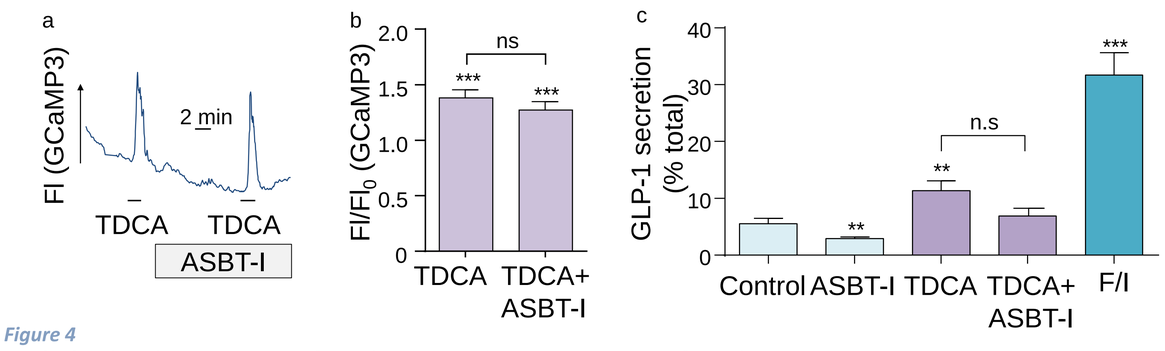
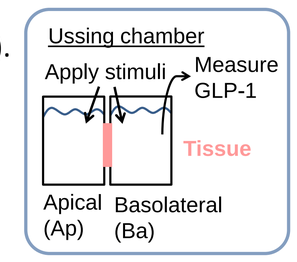



*Figure 4*
***Method:*** *(a,b) Calcium imaging performed as in Fig. 3. (c) GLP-1 secretion performed as in Fig. 1.*
***Stimuli:*** *(a) Representative calcium response to TDCA (10 µM) ± ASBT inhibitor (ASBT-I, 10 µM). (c) Test agents were TDCA (10 µM), ASBT-I (10 µM) and 10 µM forskolin + 10 µM IBMX (F/I).*
***Analysis:*** *(b) Mean increase in GCaMP3 fluorescence over baseline ($FI/FI_0$) for cells recorded as in a. Results are means + SEM; n=4. Statistical significance from basal determined on $log_{10}$ data via one-sample t-test (***p<0.001).*
*(c) Results are means + SEM of 2-3 experiments, each performed in quadruplicate (n=8-12). Statistical differences were determined using one way ANOVA and post-hoc Bonferroni on $log_{10}$ transformed data (**p<0.01, ***p<0.001 to basal).*

## 5. Bile acids stimulate GLP-1 secretion from the basolateral side

- Basolateral stimulation by bile acids is greater than upon apical application. Supported by results in perfused rat intestine (not shown).
- Apical stimulation by TDCA is blocked by an ASBT inhibitor.
- A specific GPBAR agonist (GP-A) only stimulates from the basolateral side.



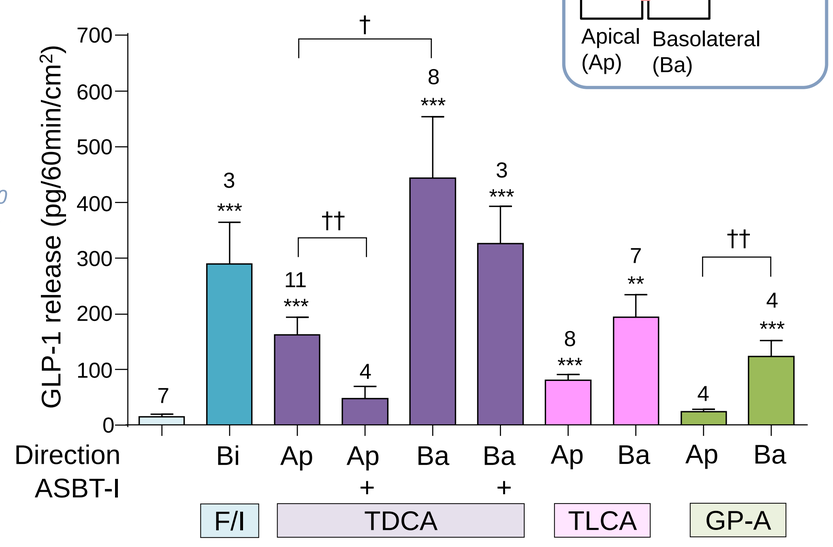




*Figure 5*
***Method:*** *Tissue segments mounted in Ussing chambers and basolateral GLP-1 concentration was assessed.*
***Stimuli:*** *(1h) Taurodeoxycholic acid (TDCA 100 µM), taurolithocholic acid, (TLCA 100 µM), GPBAR-A (GP-A 3 µM) or forskolin (10 µM) +IBMX (100 µM) (F/I), applied apically (Ap), basolaterally (Ba), or bilaterally, (Bi) as indicated. Ten minutes before application of TDCA, some tissues were pre-treated bilaterally with 10 mM ASBT-I.*
***Analysis:*** *Results are normalised per 1 $cm^2$ tissue area. Means + SEM and numbers of tissue sheets are indicated. Statistical differences were determined on $log_{10}$ data using one way ANOVA and post-hoc Bonferroni test either to basal (*) or between conditions (†) (*/†p<0.05, **/††p<0.01, ***/†††p<0.001).*

## 6. GPBAR1 is essential for bile acid stimulated GLP-1 secretion

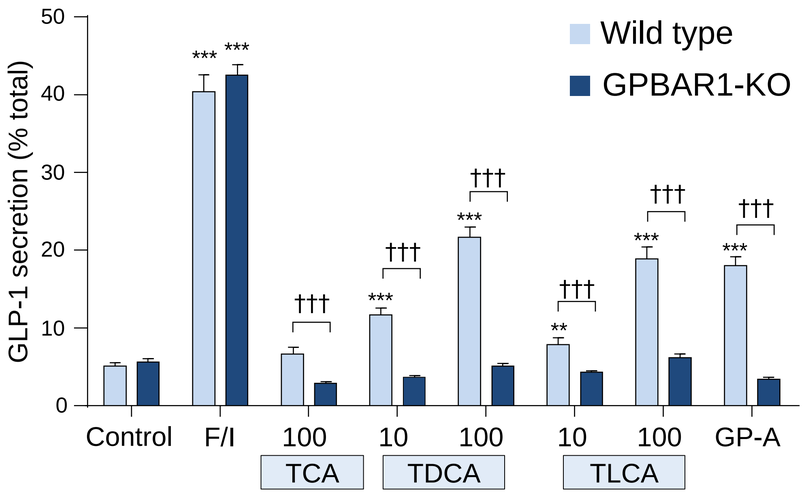



*Figure 6*
***Method:*** *GLP-1 secretion was measured as in Fig. 1. Intestinal cultures generated from wild type and GPBAR knockout mice.*
***Stimuli*** *(µM): forskolin (10 µM) + IBMX (10 µM) (F/I), taurocholic acid (TCA), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA), GPBAR-A (GP-A, 3 µM).*
***Analysis:*** *Results are means + SEM of 3 experiments, each performed in triplicate, n=9. Statistical differences were determined using a two-way ANOVA and post hoc Bonferroni test on $log_{10}$-transformed data. Statistical elevations from control (*) or differences between wild-type and knockout (†) mice are indicated: */† P<0.05, ** P<0.01, ***/††† P<0 .001.*

UNIVERSITY OF CAMBRIDGE
Metabolic Research Laboratories
Wellcome Trust – MRC IMS Institute of Metabolic Science
MRC Metabolic Diseases Unit
Supported by wellcome trust

WWW.IMS.CAM.AC.UK

*Working together to translate research into better health*