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Tissue distribution and pharmacological characterization of bovine free fatty acids-sensing GPCRs

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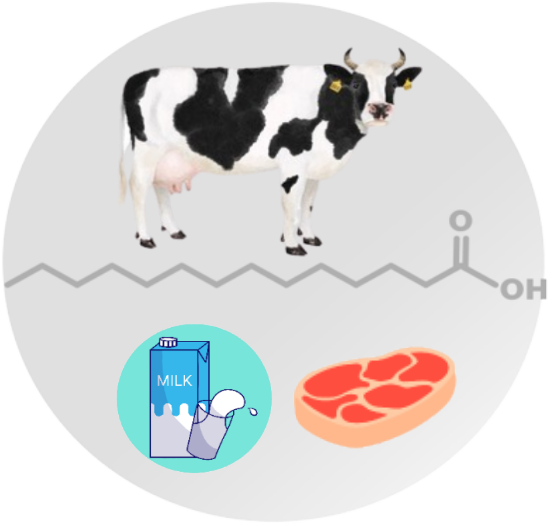
Introduction

Fatty acids (FA):

- Growth, production, and metabolism adaptation (e.g., peripartum)

- **More than energy sources**
- **Signaling molecules** ➡ regulation of metabolic functions



- Binding to different receptors:
  - SREBP, PPARs, liver X receptor (LXR) – nuclear
  - TLR4, TLR2, CD36 – cell membrane
  - G protein-coupled receptors

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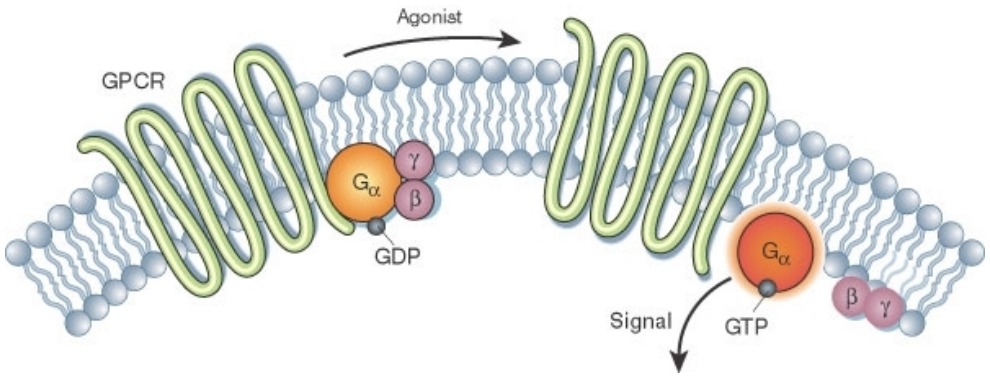
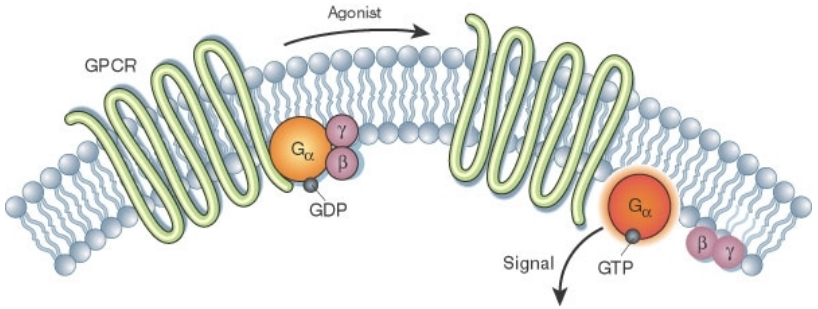


Introduction

G protein-coupled receptors (GPCR):

- Many GPCRs are activated by FFA
- FFAR1, 2, 3 and 4, GPR84



Humans and mice

- Tissue distribution/pharmacological properties
- Activation of signaling pathways and their associated biological outcomes:
  e.g., insulin secretion, inflammation, lipolysis
- FFAR as pharmacological targets for the treatment of different diseases

Dairy cows

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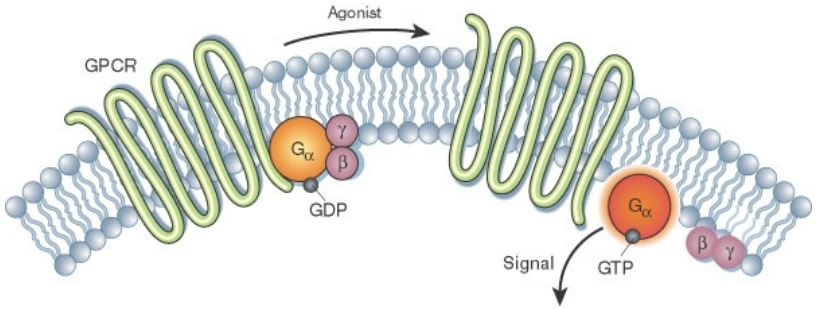

Humans and mice

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Dairy cows

- Scarce information
- Gene expression on different tissues

Objectives

- The objective of our study was to characterize 5 bovine FFARs (FFAR1 to 4, and GPR84) in regards of tissue distribution

- Moreover, we aimed to characterize the pharmacological properties of FFARs (FFAR1 and FFAR2)

Materials and methods

Tissue distribution:

- 16 Charolais bulls (16-18 months old)
- Samples from 6 tissues:
  - Liver
  - Ileum
  - Rectum
  - Spleen
  - Longissimus thoracis (LT)
  - Perirenal adipose tissue (PRAT)
- Total RNA was extracted and gene expression assessed by RT-qPCR

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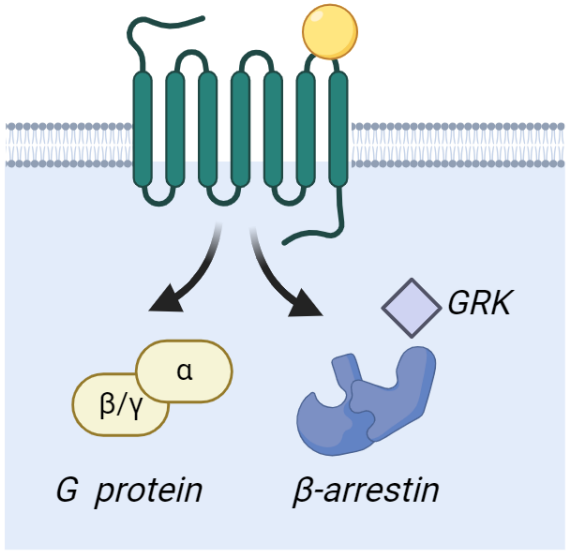
Materials and methods

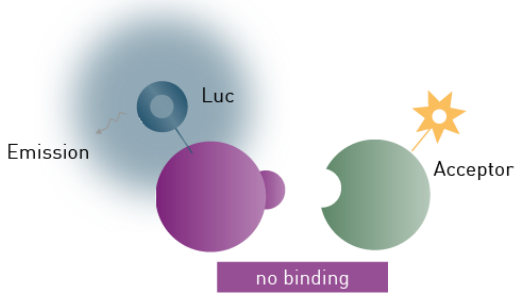
Pharmacological properties:

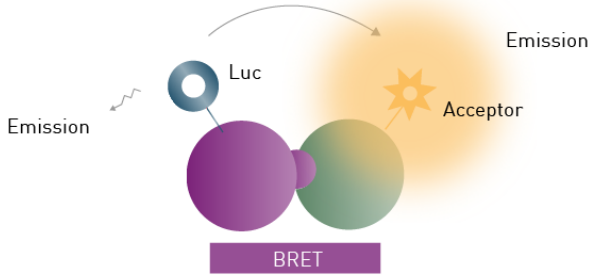
- HEK293a cells
- FFAR transfected individually
  - mG proteins (mGq, mGi, mG12, mGs)
  - B arrestin



Unbiased ligand: G = B
Bias ligand: G > B or G < B

- Bioluminescence Resonance Energy Transfer (BRET)



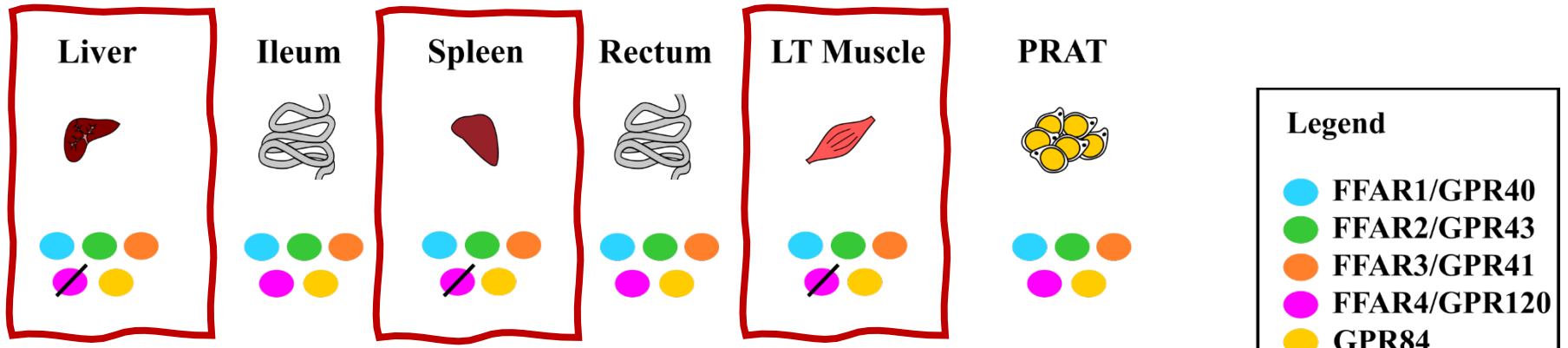


Results

Tissue distribution:

- FFAR1, FFAR2, FFAR3 and GPR84 – expressed in all studied tissues
- FFAR4 expression was restricted to ileum, rectum, and PRAT

No expression of FFAR4



FFAR mRNA tissue expression in bovine.

Results

Pharmacological properties:

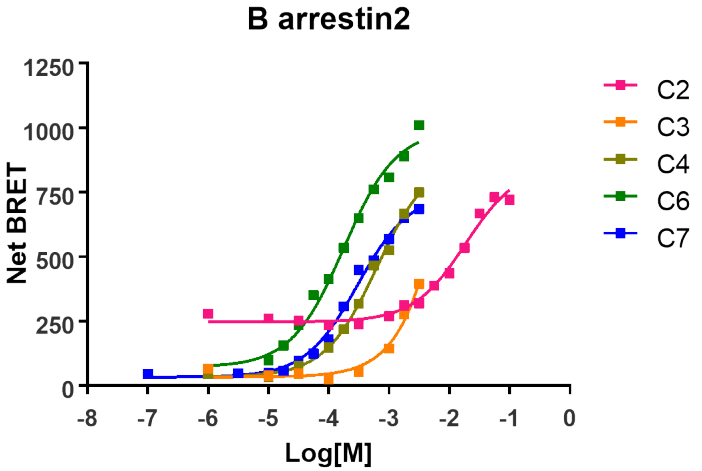
Potency (-logEC50 ± SEM) of SCFA in G proteins and B arrestin signaling in bovine FFAR2.

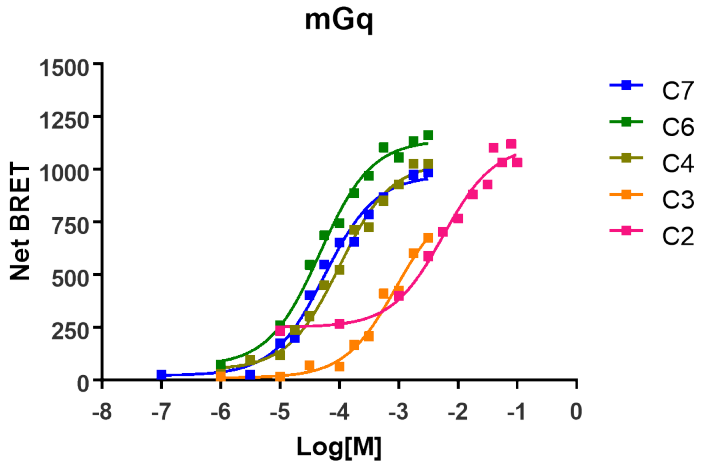
Fatty acid	mGi	mGq	B arrestin 2
C2	1.961 ± 0.07	1.961 ± 0.07	1.729 ± 0.09
C3	2.211 ± 0.07	2.211 ± 0.07	1.661 ± 0.06
C4	3.906 ± 0.06	3.906 ± 0.06	3.035 ± 0.06
C6	**4.036 ± 0.07**	**4.036 ± 0.07**	**3.842 ± 0.06**
C7	3.901 ± 0.08	3.901 ± 0.08	3.518 ± 0.07
C8 and C10	-	-	-

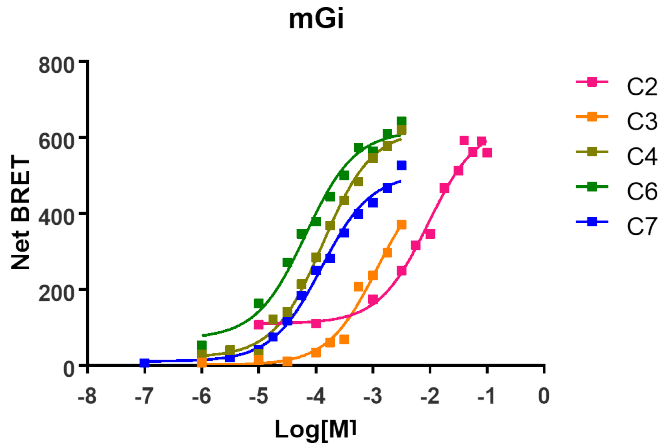
- C8 and C10: Did not showed a typical dose-response curve

Results

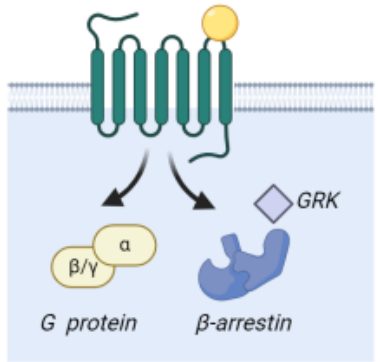
Pharmacological properties:







- C6: Greatest efficacy (Emax) and potency (-logEC50)
- No bias observed for SCFA in activating mGq, mGi or B arrestin 2 signaling



Unbiased ligand: G = B

Results

Pharmacological properties:

- LCFA conjugated or not with BSA (4:1 molar ratio)
- Fatty acids from 7 to 22 carbons
- G protein recruitment restricted to mGq:

C22:6/BSA: Greatest potency (EC50) ➡ Omega-3, Anti-inflammatory and antioxidant properties

C18:3 (ALA): Greatest efficacy (Emax) ➡ Omega-3, Essential fatty acid

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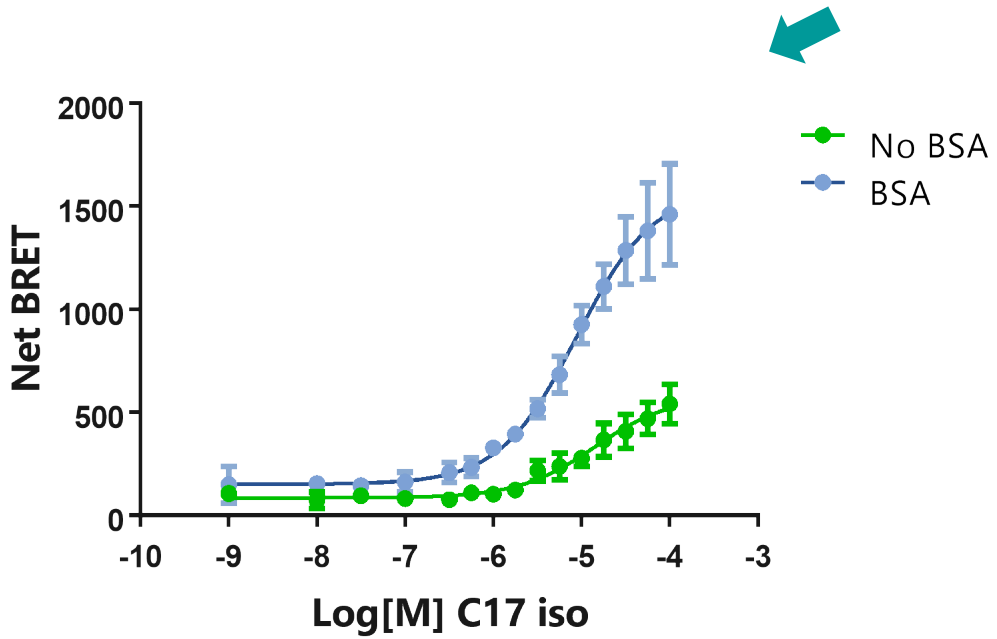
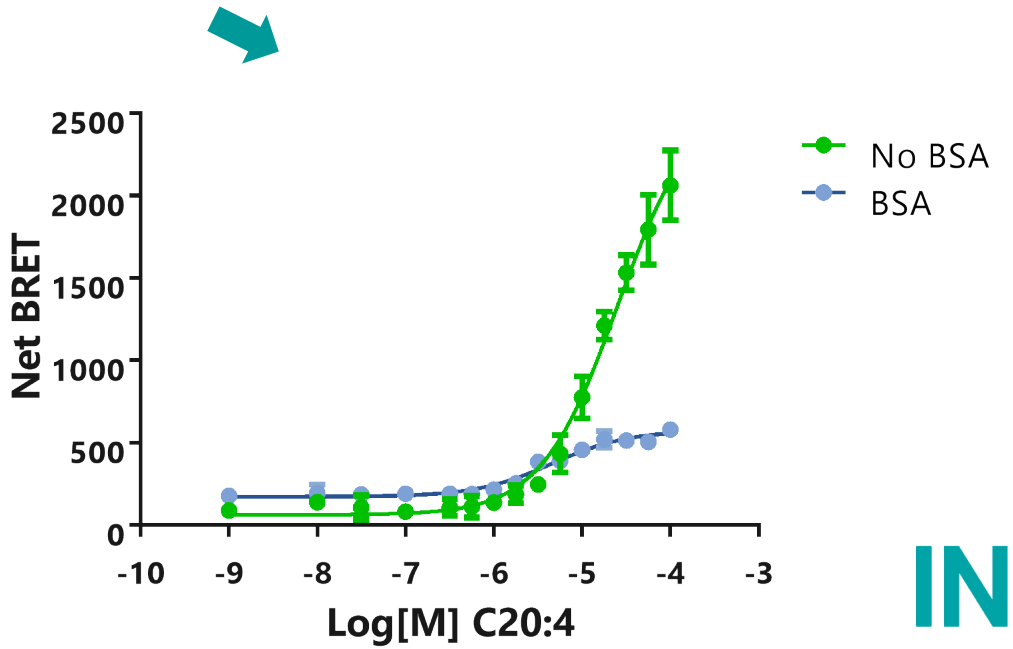
Results

Pharmacological properties:

- Efficacy (Emax) and potency (-logEC50) affected by FFA and BSA

- Efficacy (Emax): Either increased or decreased by BSA conjugation





Conclusions and Future Perspectives

Bovine FFAR2:

- Greater potency in SCFA with more carbons (C6)
  - Contrast to what is observed in humans (C2)

Bovine FFAR1:

- Conjugation with BSA affects FFAR1 response: Possible implications?
  - Activation of receptors in the GI tract by dietary FFA
  - Periods of alterations in FFA:albumin ratio (e.g., during transition period)

Conclusions and Future Perspectives

Future perspective:

- Assess B arrestin recruitment of FFAR1
- Determine pharmacological properties of the others bovine FFAR

- Further understand the biological outcomes associated with the activation of FFARs in cattle
- Possible association with, *e.g.*, metabolic disorders, adaptation around parturition?

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Thank you!