Conjugated Bile Acids Regulate Hepatic Gluconeogenic Genes via G alpha i Protein Coupled Receptor(s)

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**Hepatocyte Cell Signaling Pathways Activated By Bile Acids**

Fas-R, FGF-R4, TNF-R, TRAIL-R

Receptor Homo- and Heterodimerization interactions

- **ErbB1**
- **ErbB2**
- **ErbB3**

Rac/Rho/Cdc42 → K-Ras H-Ras → Raf-1 → B-Raf → MEK1/2 → ERK1/2 → p90 rsk → c-Jun → CYP7A1

- **MEKK1/2/3**
- **MKK4/7**

- **JNK1/2** → ERK1/2 → (active) GS → GS-P → (inactive)

- **MEK1/2** → (active) Ets → CREB → C/EBPβ

- **PI3K**
- **p110** → **p85** → **p110** → **p85**

- **PDK1** → **Akt1/2** → **GSK3**

Glycogen → UDP-glucose
Experimental Objectives

1. Characterize the regulation of gluconeogenic genes and SHP by taurocholate (TCA) in primary hepatocytes and the chronic bile fistula rat.

2. Determine if induction of SHP by TCA is linked to the activation of the insulin signaling pathway in primary hepatocytes.
Methods

Primary Hepatocytes

1. Primary rat hepatocytes were prepared by the method of Bissel and Guzelian and cultured in a chemically defined medium.

2. mRNA levels of PEPCK, G6Pase, and SHP were determined by RT-PCR.

3. Phospho-ERK1/2, phospho-AKT, phospho-GSK-3 levels were determined by SDS-PAGE and immunoblotting procedures.
Methods (continued)

Chronic Bile Fistula Rat Model

1. Chronic bile fistulas were established as previously described and bile was allowed to drain out for 48 hrs.

2. TCA was infused intraduodenally at a rate of 36 μmoles/100g rat/hour. Animals were harvested at hourly intervals, liver extracts prepared and assayed for p-AKT, p-ERK, p-JNK, p-38 and glycogen synthase activity. mRNA was extracted and assayed for PEPCK, G-6-Pase and SHP by RT-PCR.
Activation of Cell Signaling Pathways by TCA in the Chronic Bile Fistula Rat
Activation of the AKT Pathway in the Chronic Bile Fistula Rat by TCA
Activation of Glycogen Synthase Activity by Bile Acids in Primary Hepatocytes
Bile Acids Enhance Insulin Activation of Glycogen Synthase Activity in Primary Hepatocytes

Fold alteration in Glycogen Synthase activity

VEH  insulin  DCA  TCA  DCA + insulin  TCA + insulin
Genes Regulating Gluconeogenesis in Hepatocytes

Glycolysis
- ATP
- hexokinase
- ADP

Gluconeogenesis
- P_i
- glucose 6-phosphatase
- H_2O

Fructose 1,6-bisphosphate
- P_i
- fructose 1,6-bisphosphatase
- H_2O

Dihydroxyacetone phosphate

(2) Glyceraldehyde 3-phosphate
- (2) P_i
- (2) NAD^+
- (2) NAD^+
- (2) NADH + H^+

(2) 1,3-Bisphosphoglycerate
- (2) ADP
- (2) ATP

(2) 2-Phosphoglycerate

(2) Phosphoenolpyruvate
- (2) GDP
- PEP carboxykinase
- (2) GTP
- (2) Oxaloacetate
- (2) ADP
- Pyruvate carboxylase
- (2) ATP

(PEPCK)

(G-6-Pase)
Effect of TCA on PEPCK1 and G6Pase mRNA Expression in Chronic Bile Fistula Rats
PEPCK mRNA Expression after Treatment with TCA (50 µM) and Insulin (50 nM) in Primary Hepatocytes
Down-regulation of G6Pase mRNA Levels by TCA is Blocked by Pertussis Toxin in Primary Hepatocytes
Is the Induction of SHP, an FXR Target Gene, by TCA Linked to Activation of the Insulin Signaling Pathway?
AKT Activation and SHP mRNA Induction in the Bile Fistula Rat by TCA
Activation of the Insulin Signaling Pathway by TCA

DCA → TCA

Bile Acid Activated Gi-coupled Receptor

Giα subunits

GTP & GDP

PTX

O₂

Src → EGFR

EGFR

PP2 → AG1478

PTP

IRS-1

PI₃K

PI₃K

P

FOX 01

Gluconeogenic Genes

PEPCK, G-6-Pase

Gluconeogenic Genes

PEPCK, G-6-Pase

P-AKT

GSK3

Glycogen Synthase

Glycogen Synthase

Membrane

Plasma Membrane

Mit.
Pertussis Toxin Prevents the Induction of SHP by TCA in Primary Rat Hepatocytes
Wortmannin Prevents the Induction of SHP by TCA in Primary Rat Hepatocytes

Graph showing the induction of SHP mRNA (% of control) over time (hours) for DMSO + TCA (50uM) and Wortmannin + TCA (50uM).
ERK Inhibitor Fails to Inhibit SHP mRNA Induction by TCA in Primary Rat Hepatocytes
Model for How TCA Controls SHP mRNA and Glucose Metabolism in Hepatocytes.

DCA → TCA → GTP & GDP → Giα

Bile Acid Activated Gi-coupled Receptor

PTX → O2

Src → EGFR → AG1478

PTP → PIP3 → PI3K

Wortmannin

FXR → *FXR-P → SHP Promoter 

(TCA) → SHP mRNA → Glycogen Synthesis, Glycogen Synthase, GSK3

Akti-1/2 → FOX 01 → Gluconeogenic Genes (PEPCK, G-6-Pase)
Regulation of Hepatic Glucose, Lipid and Bile Acid Metabolism by Bile Acids (BA)

- Glucose
- Conjugated BA
- Conjugated or Free BA
- p-AKT
- GSK3
- GS
- Glycogen
- Glycogenolysis
- Gluconeogenesis
- Fox01:SHP
- Pyruvate
- Ac-CoA
- FFA
- Chol.
- SHP
- CYP7A1
- BA
- FGF-15/19 (Intestinal Factor)
- Conjugated or Free BA
- p-JNK
- p-AKT
- p-AKT
- *SHP
- FXR
- GSK3
- GS
- Glycogen
- Glycogenolysis
- Gluconeogenesis
- Fox01:SHP
- Pyruvate
- Ac-CoA
- FFA
- Chol.
- SHP
- CYP7A1
- BA
- FGF-15/19 (Intestinal Factor)
Summary and Conclusions

1. Bile acids activate the AKT pathway and function in a manner similar to insulin in regulating hepatic glucose metabolism.

2. The activation of the AKT pathway is required for TCA induction of SHP in hepatocytes.

3. TCA activated AKT pathway and FXR appear to be physiologically linked and probably function as one system in hepatocytes.
Our Research Team

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