Expression of GPR109A in dairy cattle immune cells

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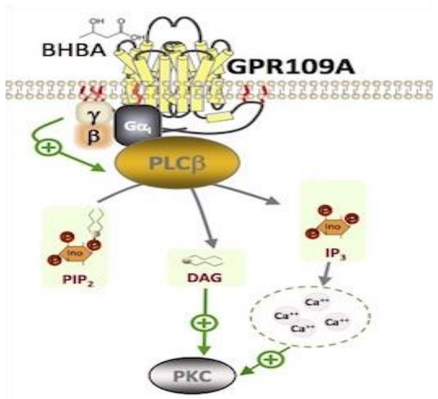
Introduction

GPR109A is a Gi- coupled receptor for nicotinic acid (NA; niacin) as well as β-hydroxybutyrate (BHB; Titgemeyer et al., 2011). In humans, GPR109A is commonly found in adipose tissue and in immune cells, but in cattle, it is found in immune cells, liver, and in various parts of the brain. In cattle, NA is known to suppress the breakdown of adipose triglycerides, primarily by acting on the GPR109A receptor. Niacin binding activates the GPR109A receptor and leads to a decrease in cyclic AMP concentrations inside the cell. The decreases in cAMP lead to the inactivation of the protein kinase A and that causes a decrease in phosphorylation of hormone sensitive lipase, which in turn reduces lipolysis. Dairy cows in early lactation have high concentrations of BHB, which may have important effects on immunity via GPR109A signaling. However, it remains unknown to what extent this receptor is expressed by key immune cells during this period of time.



Objective

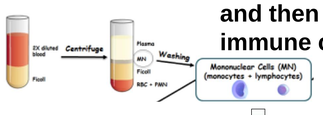
The objective of this study was to determine the relative mRNA abundance and protein expression of GPR109A in immune cells of periparturient dairy cows.

Materials and Methods

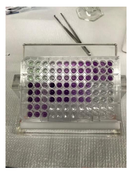
Healthy multiparous Holstein cows

Blood samples were acquired from the jugular vein of healthy multiparous Holstein cows.

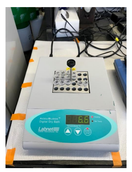
Immune Cells: Isolation



Ficoll was added to blood samples and then centrifuged to isolate immune cells.



Pierce BCA Total Protein Assay Kit (Thermo Scientific, cat#23227) was used to determine the concentration of the protein concentration in immune cell samples.



Samples were prepared for the Western Blot procedure using sample buffer and heat shocking them at 95 °C.

Gel electrophoresis was set up and 40 µg of sample were loaded into the wells and ran.

The proteins on the gel were then transferred onto a nitrocellulose membrane using the iBLot 2 system.

The membrane was blocked with a blocking solution consisting of 5% dry milk and 1% BSA, to get a block on the non-specific proteins.

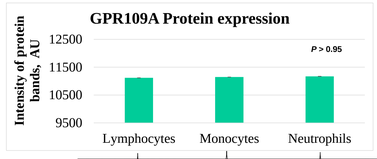
After blocking, the membranes were washed and incubated with primary antibody overnight at 4°C on a rocker (rabbit polyclonal antibody 1:500 (HCAR2, ABClonal). The secondary anti-rabbit antibody 1:10000 was added and incubated for an hour at room temperature.

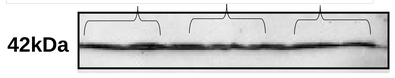
Immunodetection was performed by chemiluminescence (West-Dura; Thermo Scientific, Waltham, MA). The band images were visualized using a photodocumentation system (ChemiDoc-It Imaging System; UVP Inc., Upland, CA).

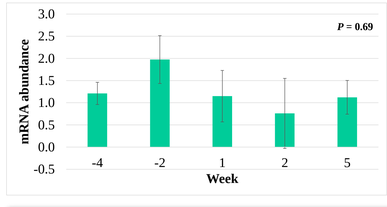
GENE	CODE #	FORWARD	REVERSE
GPR109A	XM_015475510.1	GCCGTGGATAGGTACTTCCG	ATGGTGATGCCCCACAAGAG
GAPDH	NM_001034034.2	CCGTTCGACAGATAGCCGTA	ATCCGTTCACTCCGACCTTC
RPS15	NM_001024541.2	GGCGGAAGTGGAACAGAAGA	GTAGCTGGTCGAGGTCTACG

Quantitative real-time PCR was performed to determine mRNA abundance in immune cell samples. 2 µg of total RNA per sample was used as a template for the reverse transcriptase reaction using random primers. RT-PCR was ran in duplicate using 5% of the cDNA product on 96-well plates with 200 nM of gene-specific primers and SYBR Green master mix. All target genes in samples were normalized against the control genes GAPDH and RPS15.

Results







GPR109A protein was consistently found in bovine lymphocytes, neutrophils, and monocytes from periparturient cows.

GPR109A mRNA was consistently detected in neutrophils, and there was no significant change in abundance over a 9-week period around calving.

Conclusions

GPR109A receptor is found both key types of innate immune cells (monocytes and neutrophils) of dairy cattle throughout the transition to lactation. Previously-reported associations between high blood BHB concentrations and poor immune function may be linked by GPR109A signaling.

References

Titgemeyer, E.C, et al. “An unusual distribution of the niacin receptor in cattle.” *Journal of Dairy Science*, vol. 94: 4962-4967. *doi:10.3168/jds.2011-4193*