The Effect of Ufmylation on Pancreatic Enzymes and the Secretory Function of the Exocrine Pancreas

Camille Miller

GRU GEORGIA REGENTS UNIVERSITY

Acute Pancreatitis

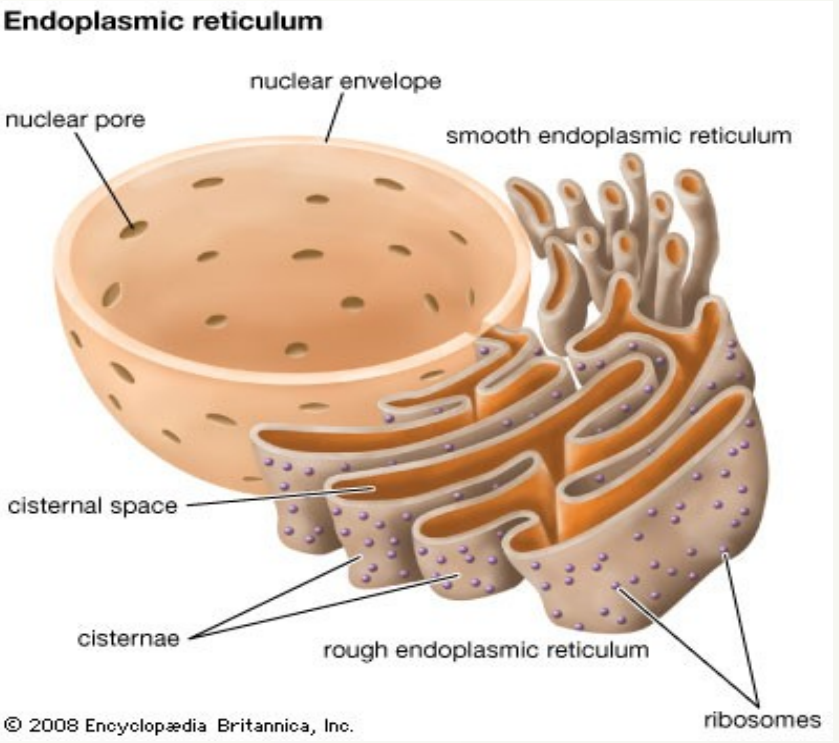
- Defined as the acute inflammation of the pancreas. It is associated with the scape of activated pancreatic enzymes from the zymogen granules into the parenchyma of the pancreas, which causes autodigestion and necrosis.

- Alcoholism is one of the causes of pancreatitis

Rough Endoplasmic Reticulum

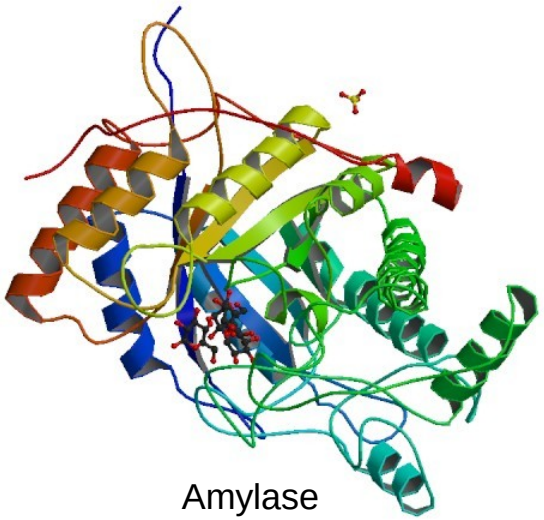
- The rough endoplasmic reticulum (RER) involved in the synthesis and folding of pancreatic enzymes.

- It is also involved in the removal of misfolded pancreatic enzymes.



Unfolded Protein Response

- The accumulation of misfolded pancreatic enzymes in the endoplasmic reticulum activates an unfolded protein response (UPR). This to apoptosis and acute p



Amylase

Ufmylation

- Ufmylation is a novel post-transitional ubiquitin-like modification system involved in unfolded protein response (UPR).

- Ufmylation consists of Ufl1 (also known as RCAD), Uba5 (also known as E1), Ufc1 (also known as E2), and poorly characterized E3 ligase.

- Since Ufmylation has been involved in RER homeostasis and a loss of homeostasis of the RER causes pancreatitis, ***there is a need for determining the participation of Ufmylation in the pathogenesis of alcoholic pancreas.***

Hypothesis

- Ufmylation causes post-translational modifications of pancreatic enzymes. These post-translational modifications are necessary for correct folding/sorting of pancreatic enzymes, and pancreatic secretion.
- If Ufmylation does not take place, pancreatic enzymes become default and are accumulated in the acinar cells, which in turn, cause ER stress, apoptosis, and finally acute pancreatitis

Aim 1

Determine the level of expression of RCAD and its ligand DDKGK1 in both normal and alcoholic pancreas of rats.

Aim 1: Materials and Methods

- Inducing alcoholism in model rats:

Control rat

Alcoholic rat

8 Weeks

Leiber-De Carli liquid diet

8 Weeks

Leiber-De Carli liquid diet containing ethanol
(36% of total calories)

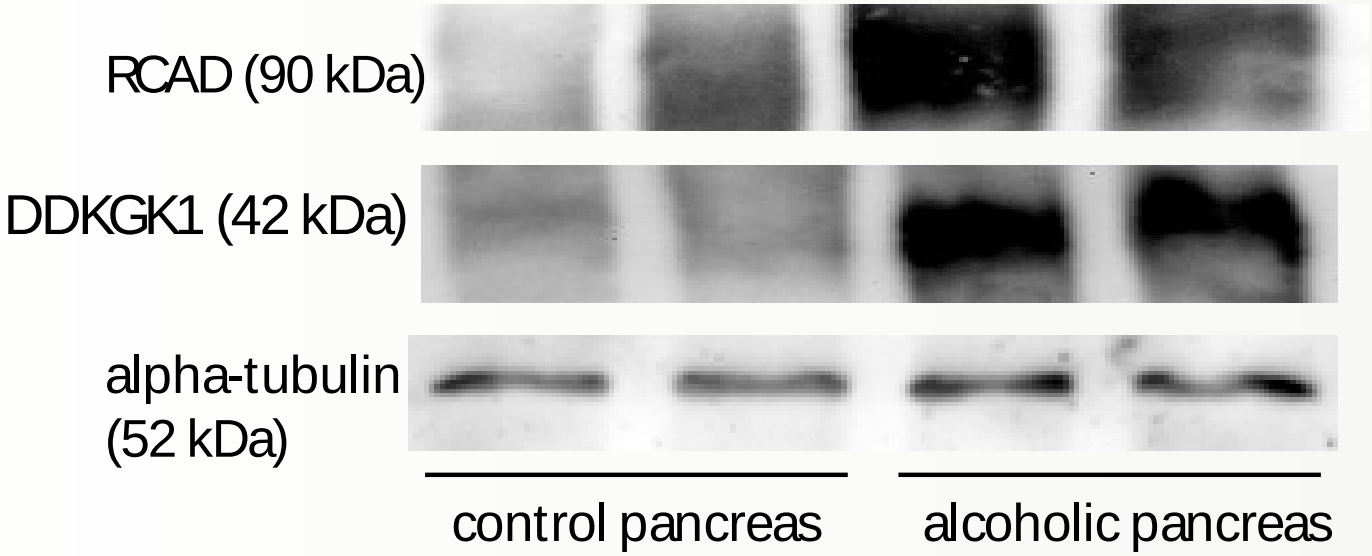
Rats were provided by Dr. Tadd Patton

GRU GEORGIA REGENTS UNIVERSITY

Aim 1: Materials and Methods

- Western-blotting:
  - 10% polyacrylamide gel
  - Nitrocellulose membrane
  - 5% BSA
  - Primary antibodies:
    - Rat anti-RCAD antibody
    - Rat anti-DDKGK1 antibody
    - Mouse anti-α tubulin antibody

The levels of RCAD and DDKGK1 increase in alcoholic rats.



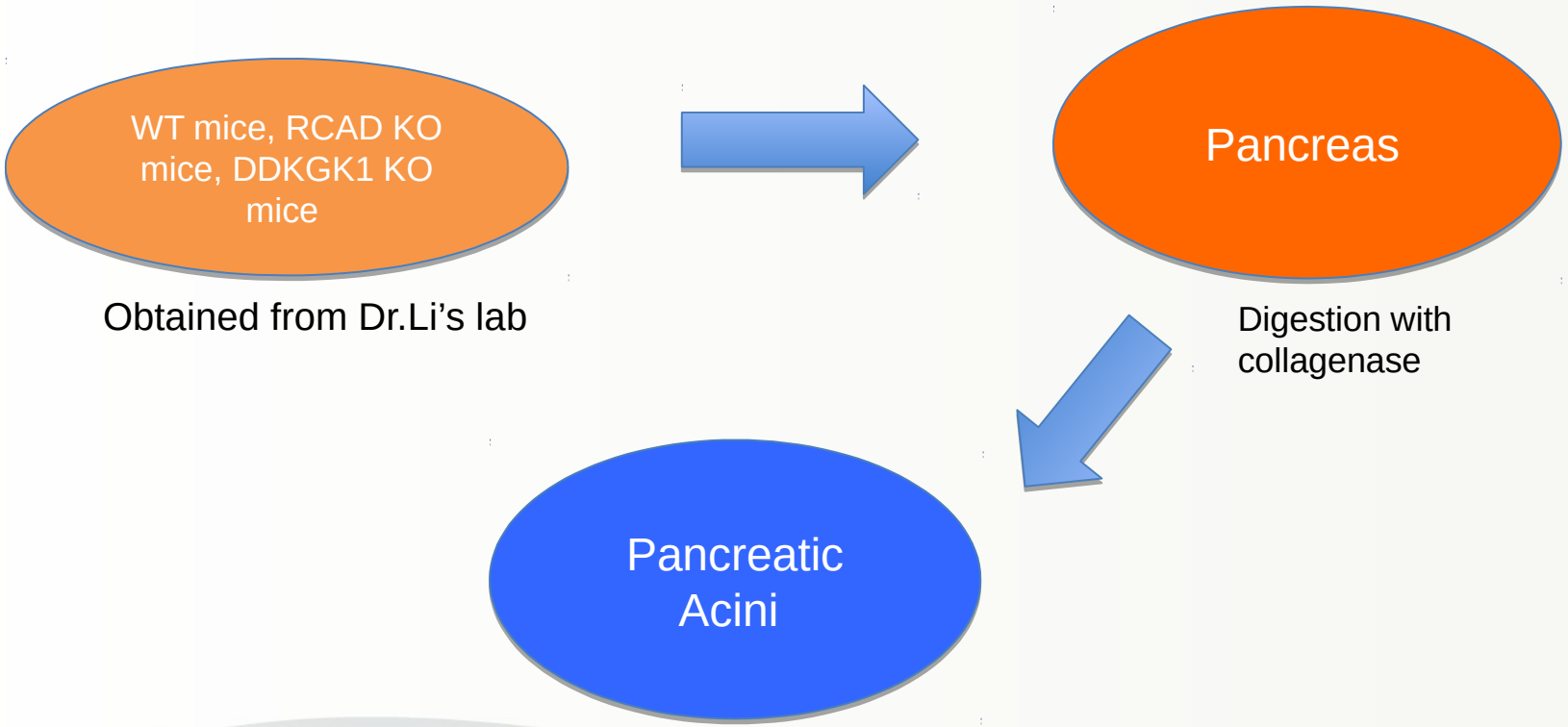
GEORGIA REGENTS UNIVERSITY

Aim 2

Determine the effect of the lack of either RCAD or DDKGK1 on amylase secretion.

Aim 2: Materials and Methods

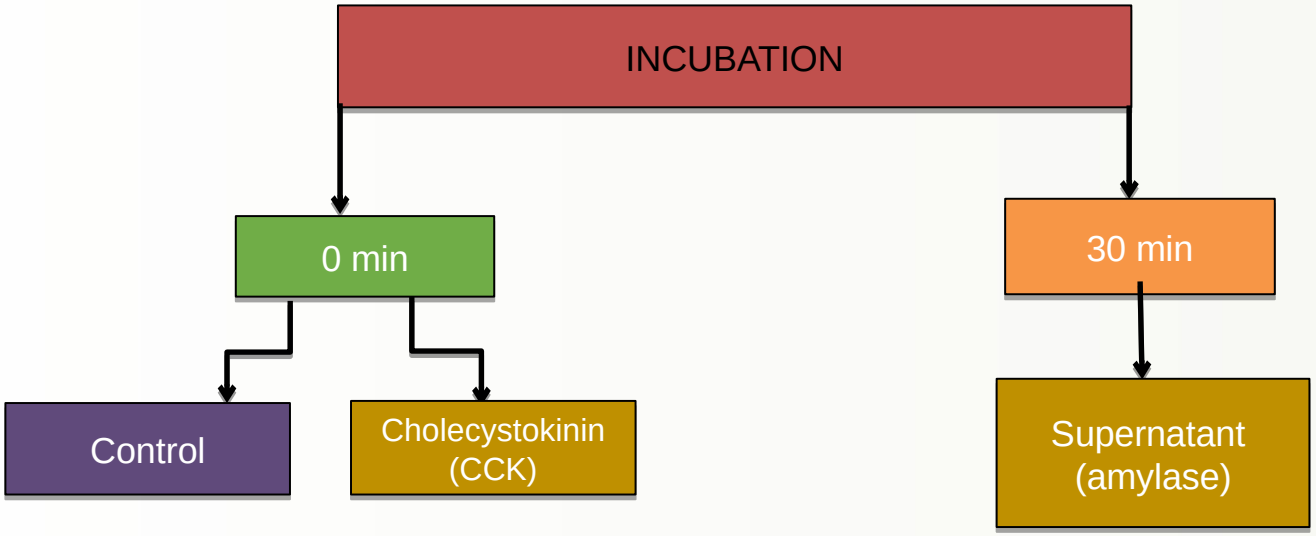
STEP 1: Isolation of pancreatic acini



GEORGIA REGENTS UNIVERSITY

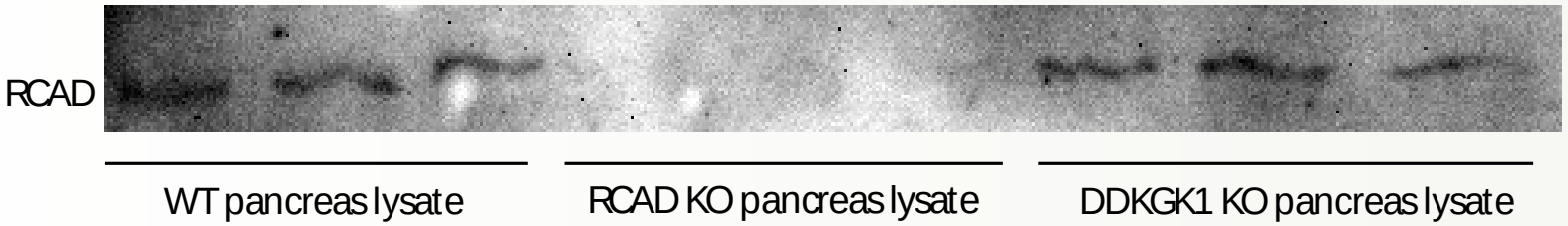
Aim 2: Materials and Methods

STEP 2: Stimulation of pancreatic acini with cholecystokinin (CCK) and determination of amylase concentration



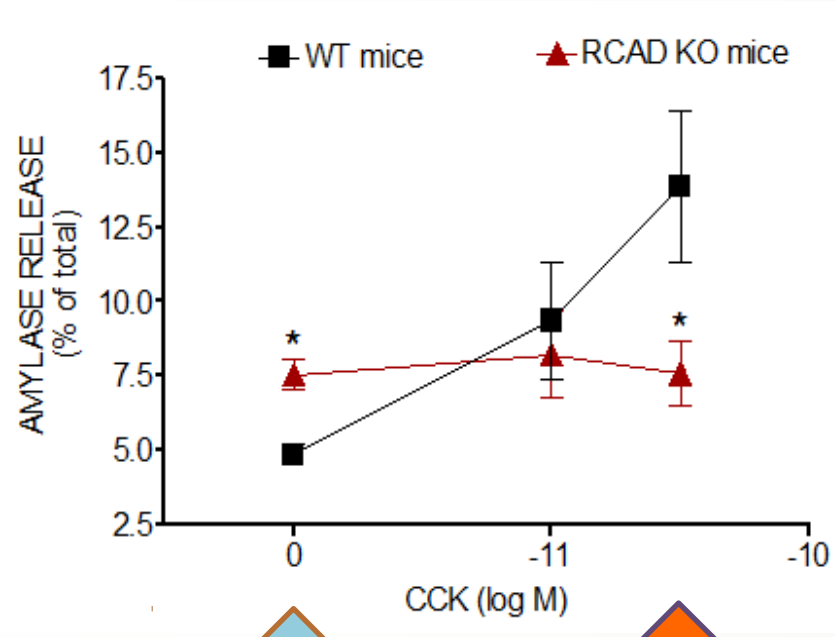
Determined by Phadebas® Amylase Test

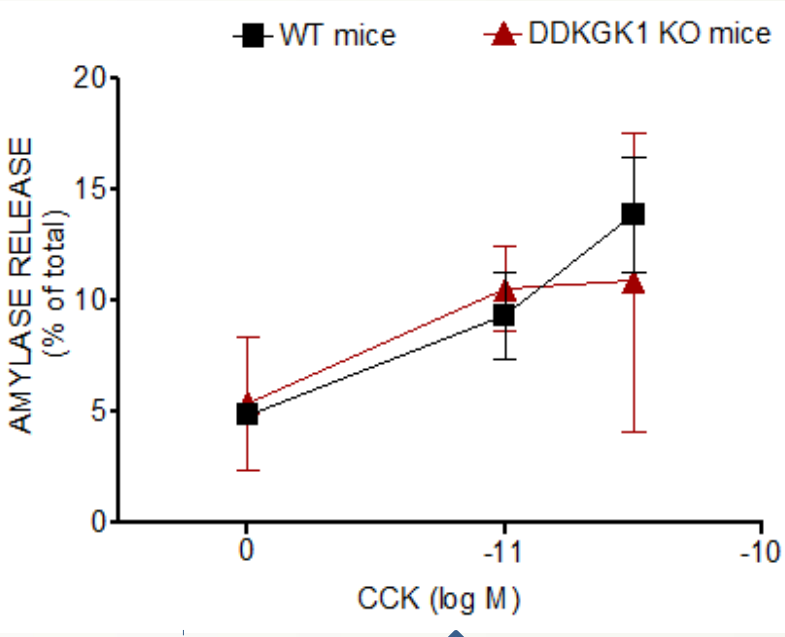
Conformation of knocking out either RCAD or DDKGK1



GRU GEORGIA REGENTS UNIVERSITY

The lack of RCAD increases the constitutive amylase secretion and impairs the effect of CCK





The lack of RCAD induces increased amylase secretion

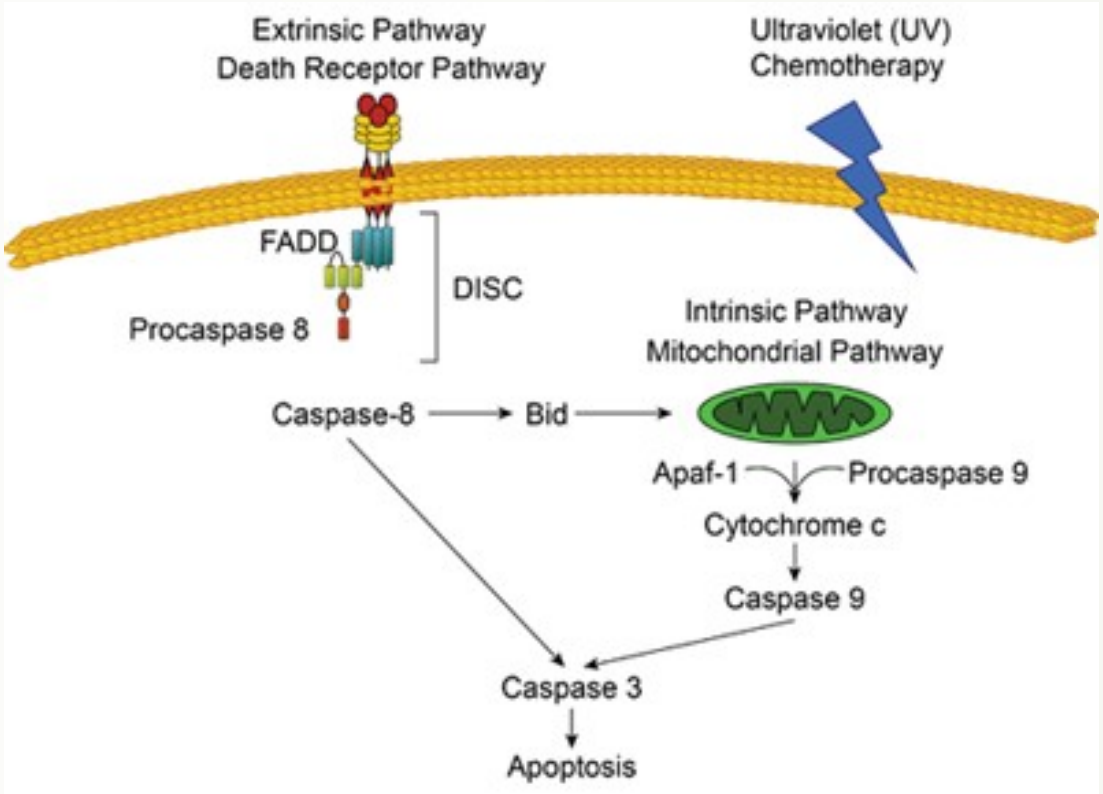
The lack of RCAD impairs CCK-induced amylase secretion

The lack of DDKGK1 does not affect either basal or stimulated amylase secretion

GRU GEORGIA REGENTS UNIVERSITY

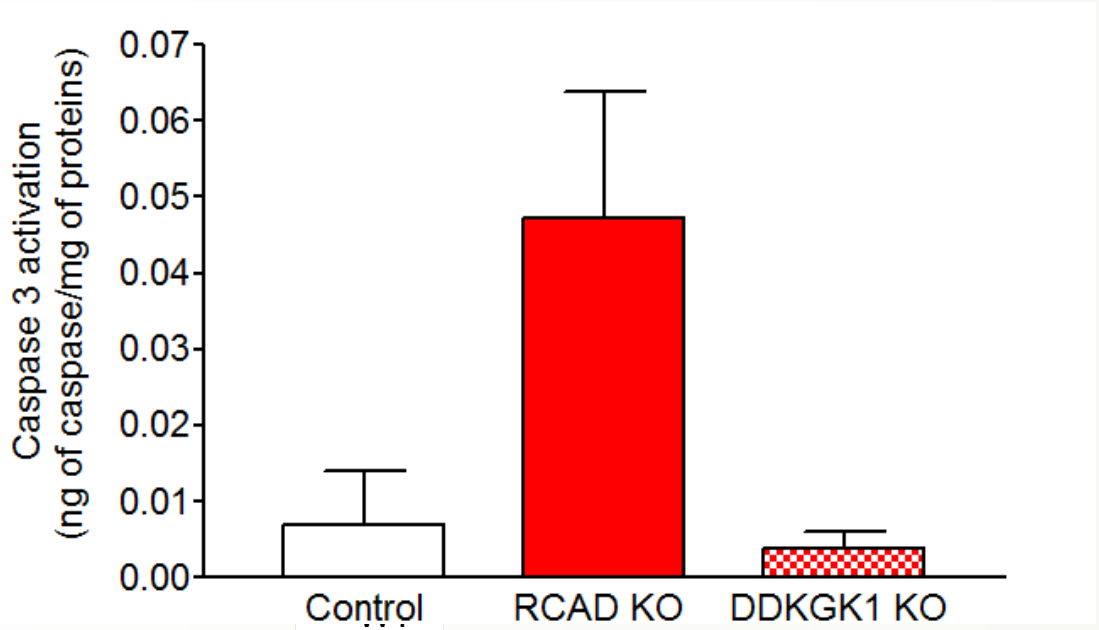
Aim 3

Determine the effect of the lack of either RCAD or DDKGK1 on Caspase 3 activation.



Aim 3: Materials and Methods

The lack of RCAD causes a small increase on Caspase 3 activatio



The lack of RCAD causes an small increase, which is not statistically significant.

Conclusion

- Both RCAD and DDKGK1 are highly expressed in alcoholic pancreas of rats.
- The lack of RCAD shows an increase in amylase secretion from pancreatic acini that has not been induced by cholecystokinin (CCK). Once the pancreatic acini has been stimulated with cholecystokinin, the lack of RCAD impairs amylase secretion.
- The lack of DDKGK1 does not affect the amylase secretion from pancreatic acini, with or without cholecystokinin stimulation. This suggests that another RCAD ligand is involved in the folding/sorting of amylase.
- The lack of RCAD causes a small increase on Caspase 3 activation.

Acknowledgements

- This work was supported by the GRU Department of Biological Sciences and CURS.

- Thank you to:
  - Dr. Li and Dr. Yafei Cai from the Cancer Center
  - Dr. Tadd Patton from Department of Psychology
  - Dr. Sabbatini

GRU GEORGIA REGENTS UNIVERSITY

Thank you for attending!

Next Brown Bag Seminar is on Friday, October 9th!

GRU GEORGIA REGENTS UNIVERSITY