Western Blot Semi-Quantitative Analysis of Non-Canonical cAMP-Dependent Protein Expression Induced by PACAP

Emily Jones
Montgomery Blair High School
Dr. Lee Eiden & Yvonne Holighaus
National Institutes of Health

Purpose

The goal of this project was to see if a hormone that prevents brain cells from dying could protect cells through a previously unknown pathway.

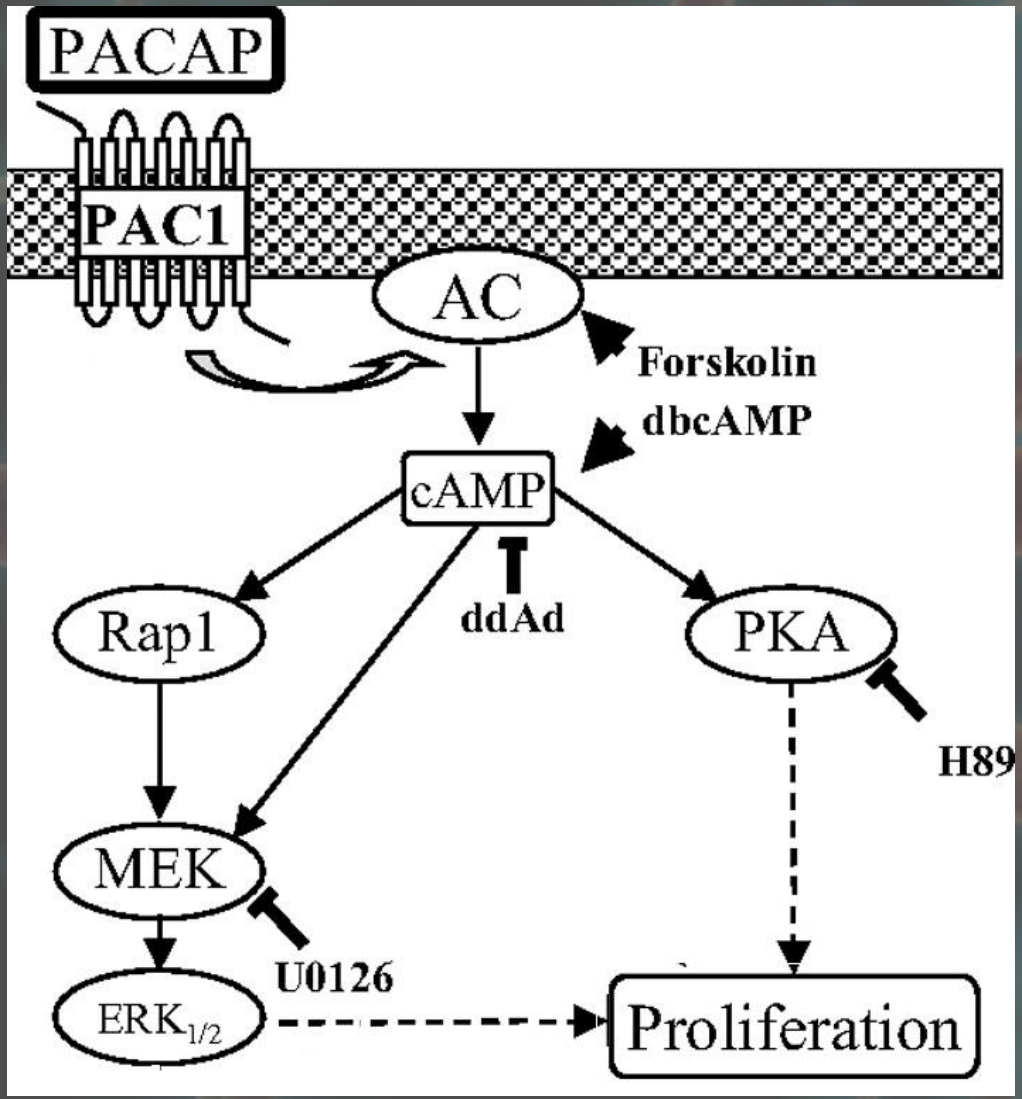
We also aimed to develop a method to determine the concentration of a certain protein in cell samples using results of a normally qualitative analysis technique.

Background: PACAP

- PACAP – pituitary adenlyate cyclase-activating polypeptide
  - Many protective functions in the central nervous system
- PACAP binds to receptor → receptor activates G-protein → G-protein activates AC → AC produces cAMP
  - Known pathway: cAMP activates PKA
  - New pathway: cAMP activates MAPKs, which activate ERK1/2

→ Genes are transcribed into proteins

Background: Signal Transduction



Adapted from Ravni et al., 2008

Background: Cerebral Ischemia

- Strokes trigger hypertoxicity
  - Elevated calcium and phosphate levels are mediators of glutamatergic death
- PACAP regulates phosphate and calcium homeostasis to prevent cell damage and death *in vivo*

Methods: Cell Samples

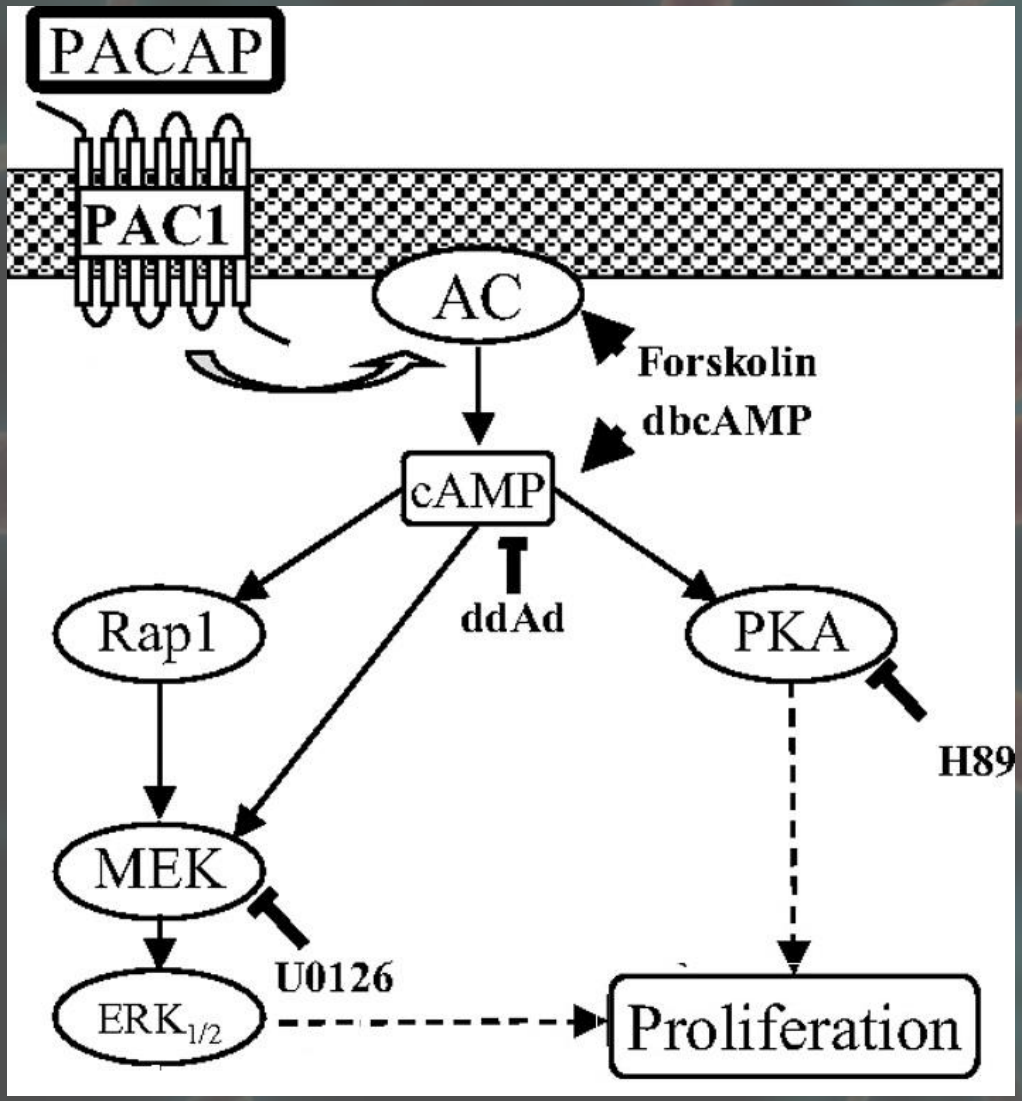
- NG108-15 and cortical cells
- Calibration cell samples:

25µL PACAP	10% dilution	20% dilution	50% dilution
75% dilution	87.5% dilution	93.5% dilution	96.8% dilution

- Pharmacology cell samples:

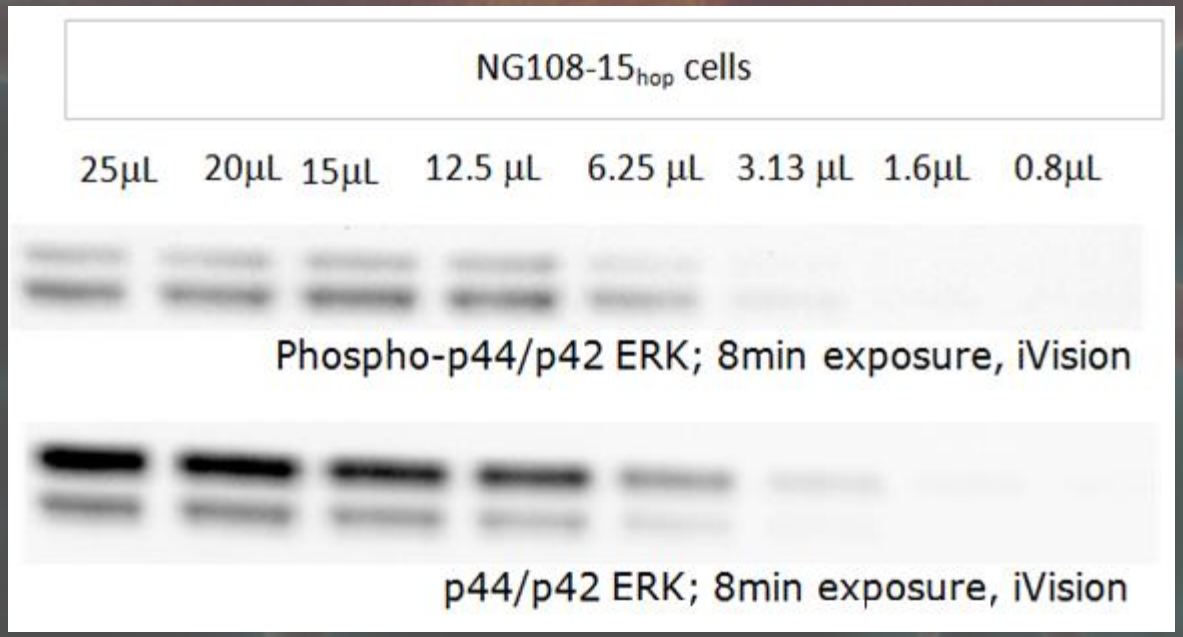
---	ddAd	H89	U0126
PACAP (or forskolin)	PACAP + ddAd	PACAP + H89	PACAP + U0126

Methods: Cell Samples



Methods: Western Blotting

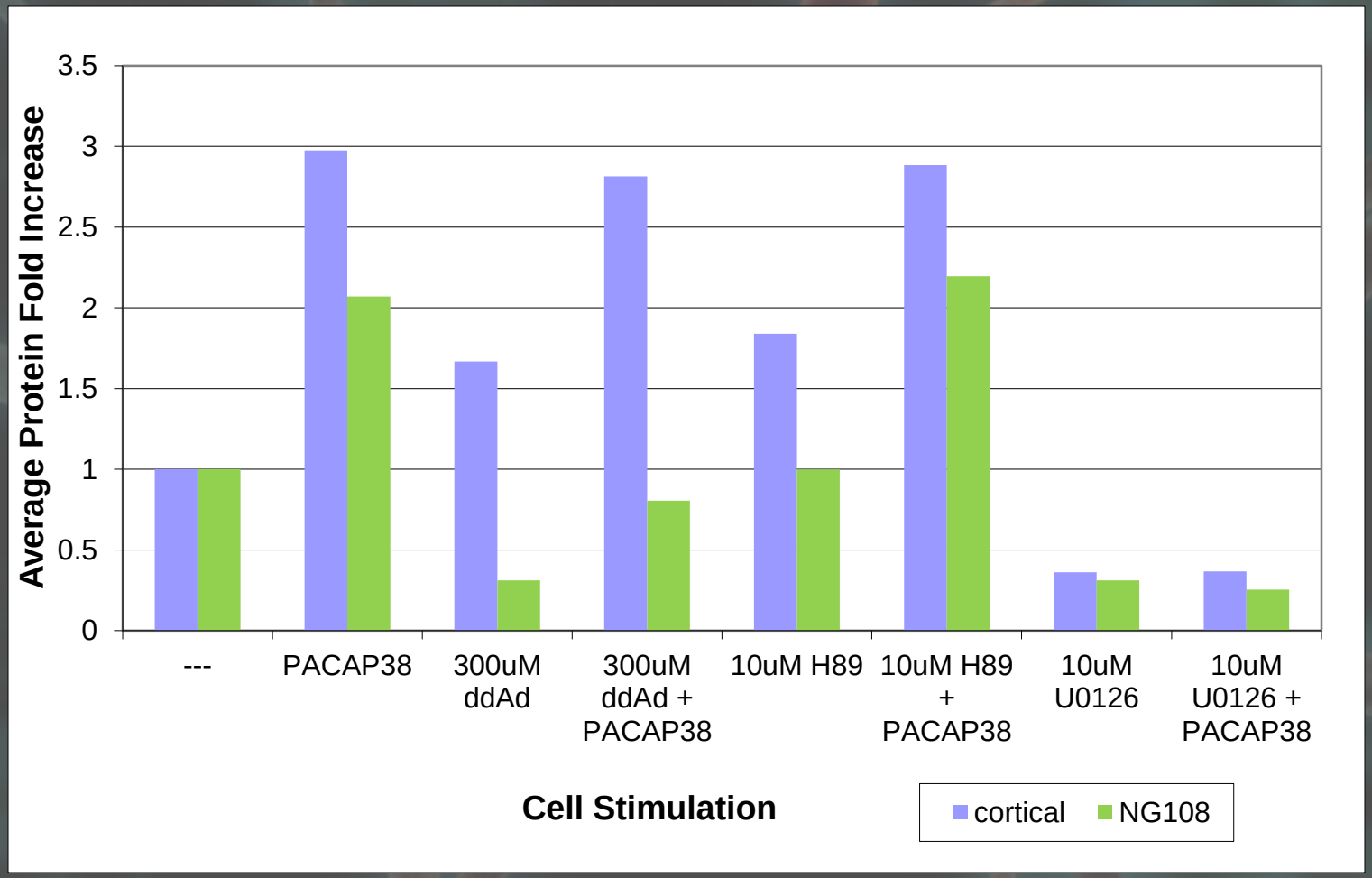
- SDS-PAGE: Separated proteins by length
- Incubated in antibodies: phospho-ERK and ERK
- Incubated in chemiluminescent substrate



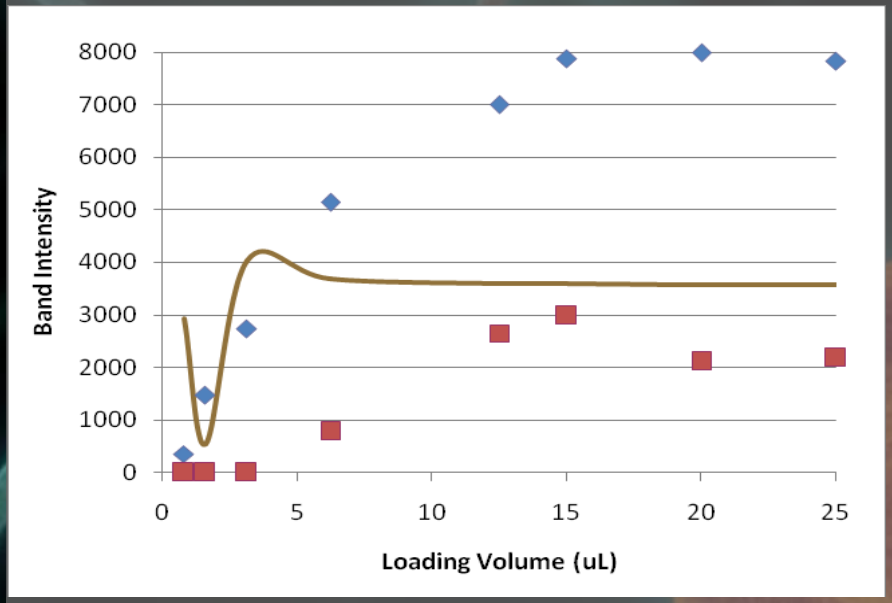
Methods: Hyperbolic Regression

- Band Intensity vs. Protein Amount is not a linear relationship
  - Background deletion corrects chemiluminescent substrate problems
  - Band intensity measured with ImageJ gel analysis tool
  - Division by loading control corrects gel loading variation
  - Calculated calibration equation via hyperbolic regression script

Results: Protein Fold Increase

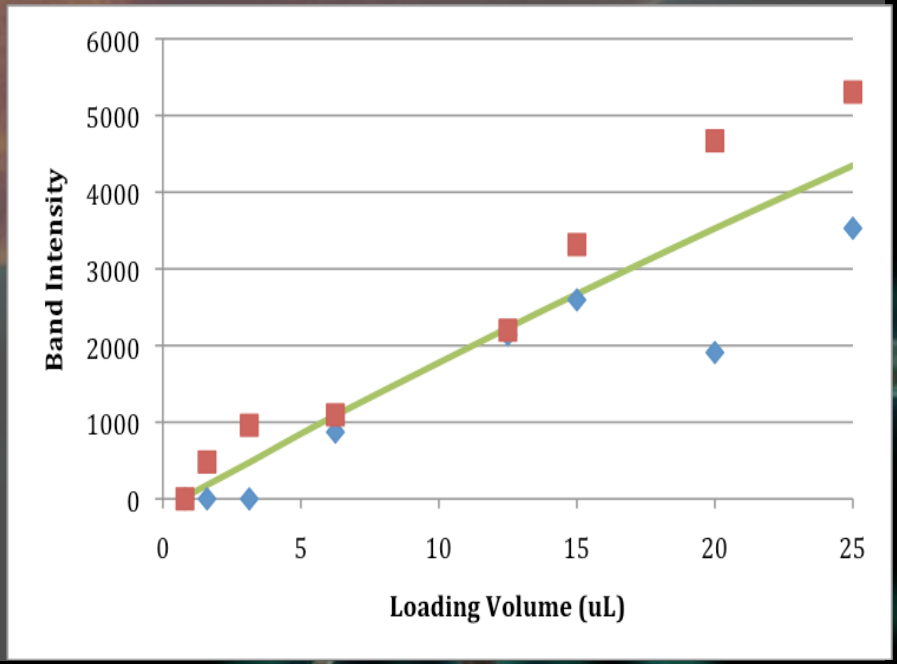


Results: Calibration Curve



NG108-15 cells

Cortical cells



Results: Calibration Curve

- Sum of errors:

NG108-15 phospho-ERK	24,287,533
NG108-15 ERK	631,011
cortical phospho-ERK	160,739
cortical ERK	1,233,319

- Inaccurate for NG108-15 phospho-ERK blots and cortical ERK blots because the saturation point for band intensities was 12.5µL
  - curve was very sensitive to fluctuations at smaller dilutions and flattened out

Conclusion

- Non-canonical pathway via ERK rather than PKA activation exists in rat cortical cells
- Analysis incomplete: did not have enough blots to correct curve due to chemiluminescent substrate difficulties
  - Background deletion problems
  - High blot-to-blot variation lead to high standard deviations

Further Research

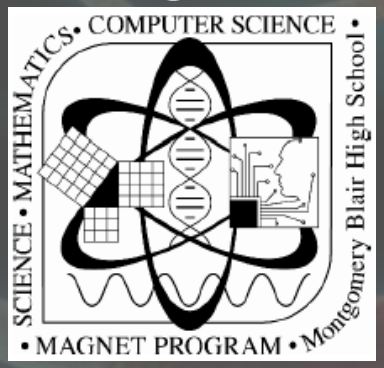
- Create calibration curve with more than two blots
- Evaluate accuracy of method using known protein concentrations

Further Research

- Upregulation of other genes via ERK pathway
  - Target gene discovered by microarray also regulates calcium and phosphate concentrations *in vitro*
- Pathway in other cells with PAC1 receptor
- Pathway could be targeted in drug development if only exists in neuronal cells
  - prevent damage during neurodegenerative disease progression or post ischemic insult

Acknowledgements

Montgomery Blair High School:



Susan Ragan

Elizabeth Duval

National Institutes of Health:

Dr. Lee Eiden

Yvonne Holighaus