Altered Vasoconstrictor signaling: Rho-kinase and PKC, new players in an old problem

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Medline Search Results mid 1960's - present

- Penile circ & Vasoconstrictors 36
- Coronary circ & Vasoconstrictors 197
- Cerebral circ & Vasoconstrictors 266
- Systemic circ & vasoconstrictors 250

- Erection/ED & Vasoconstrictors 57
- Erection/ED & Nitric oxide 624

Diabetes and ED Statistics

- > 50 % of all men with DM have some form of ED
  - (estimated 8 million US men).
- Onset of ED occurs earlier with DM
  - (usually within 10 years of DM onset).
- A variety of pathological changes are associated with DMED.
  - Psychological function
  - Central and peripheral nerve function
  - Steroid metabolism
  - Vascular function

Vascular Changes

- Reduced relaxation to ACH, NO-cGMP, or PGE1-cAMP.
- Impairment of neurogenic NO and cGMP production.
- Direct relaxation by NVDs are usually unaffected.
- Increases in expression of AGE's and iNOS.
- Altered tissue morphology.
- Altered smooth muscle responsiveness to vasoconstrictors.

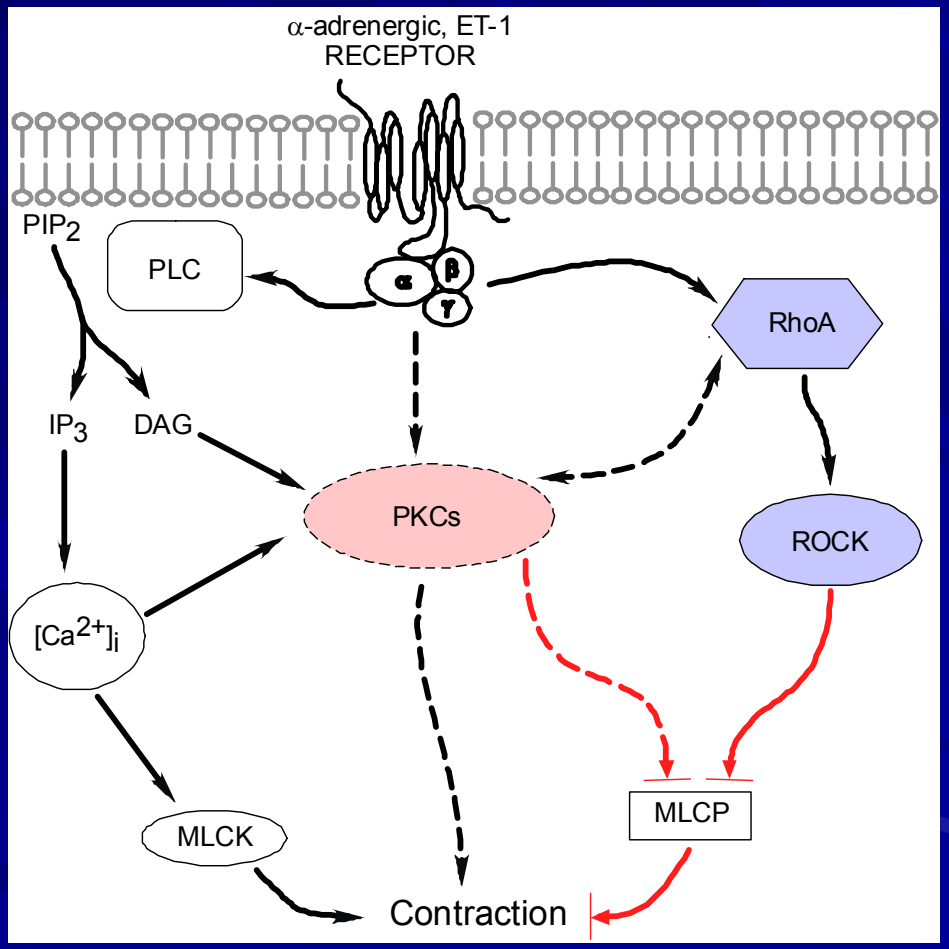
Altered Vascular Reactivity

- Impaired Relaxations:
  - Involving both endothelial-dependent and independent mechanisms.

- Elevated Constrictor Activity:
  - Increased constrictor tone through alteration in receptor levels/activity.
  - Increased action of signaling pathways.

Working Hypothesis

The erectile dysfunction observed in Zucker obese-diabetic animal model is mediated through alteration of vasoconstrictor regulation of cavernosal smooth muscle tone.

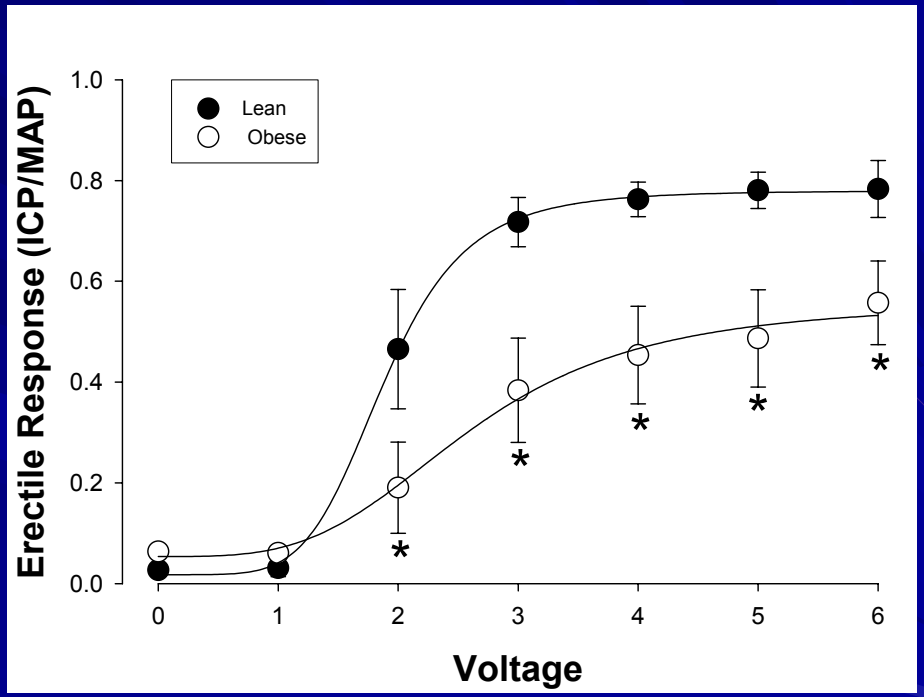


Metabolic Syndrome X

- Cluster of metabolic diseases including, insulin resistance, glucose intolerance, obesity, hypertension and hyperlipidemia.

- The Zucker rat (ZDF) is a obese, hypertensive, hyperglycemic rat with a nonsense mutation of the leptin receptor gene, (Recessive trait and displays no leptin receptor expression).

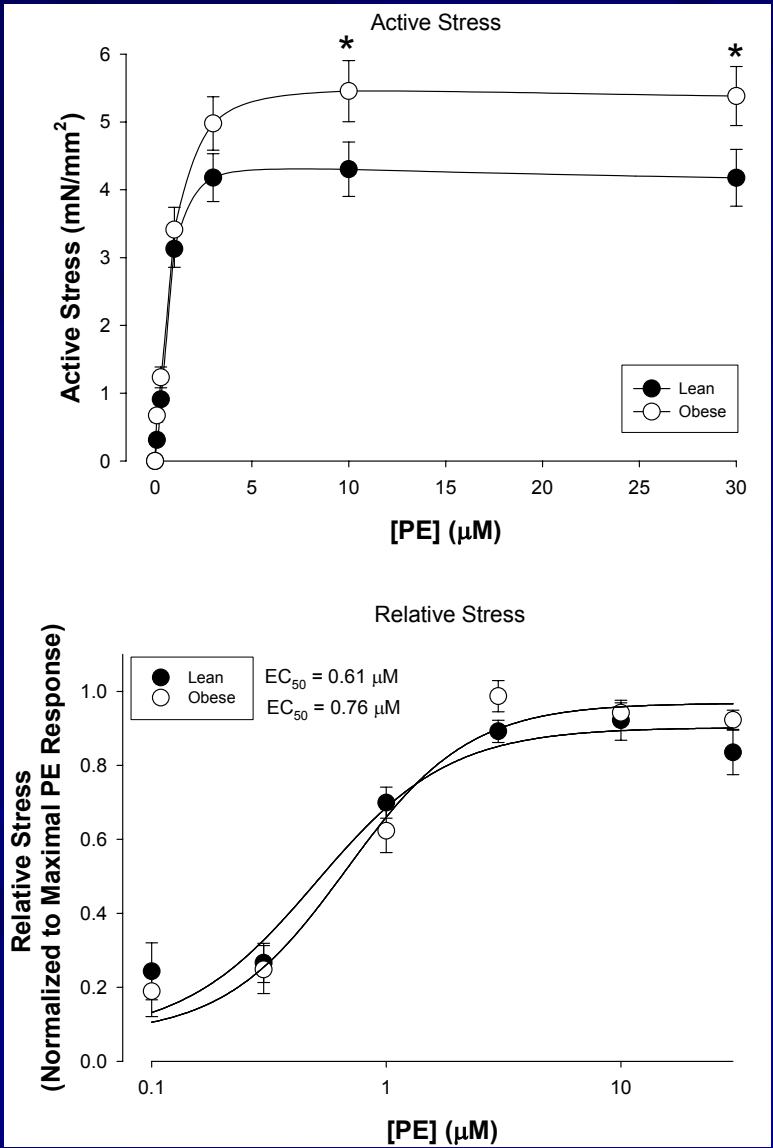
Erectile Response



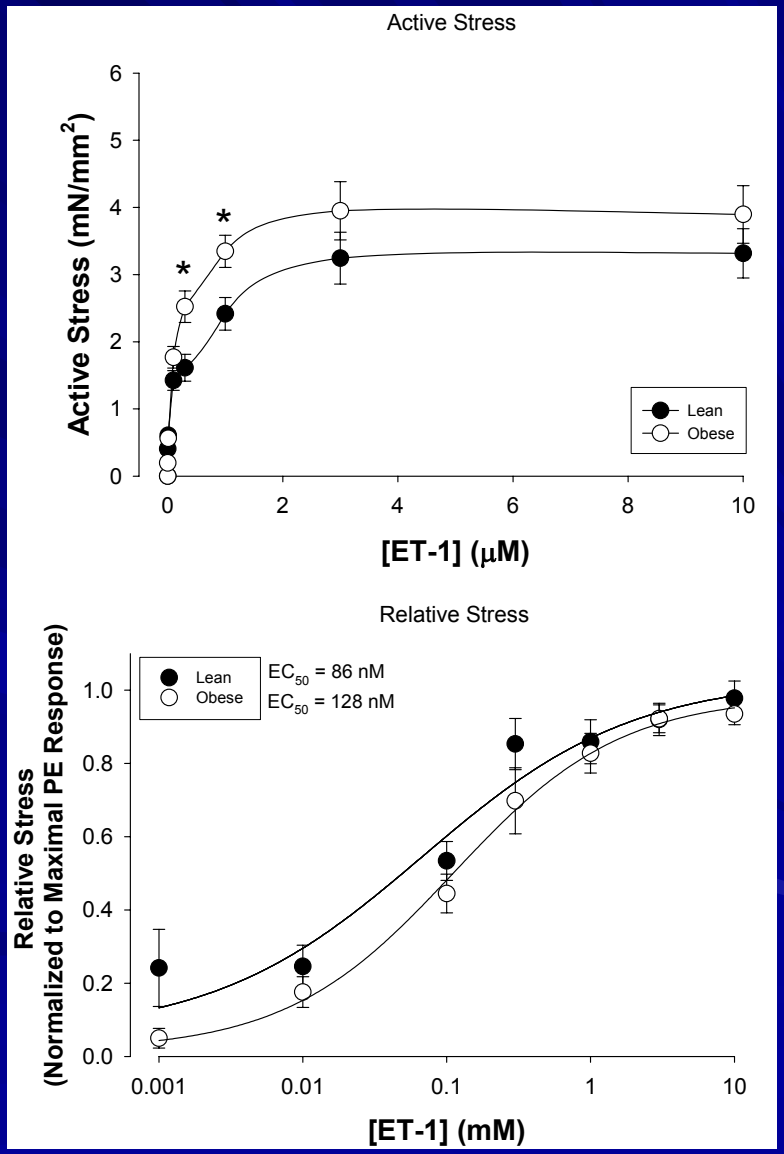
Wingard *et al.*, Physiologist, 46: 237, 2003

Contractile Responses

PE



ET-1



RhoA/Rho-kinase maintained vasoconstriction in diabetic tissues

- Vascular tissues contain RhoA & Rho-kinase.

  – **Vascular tissues:**

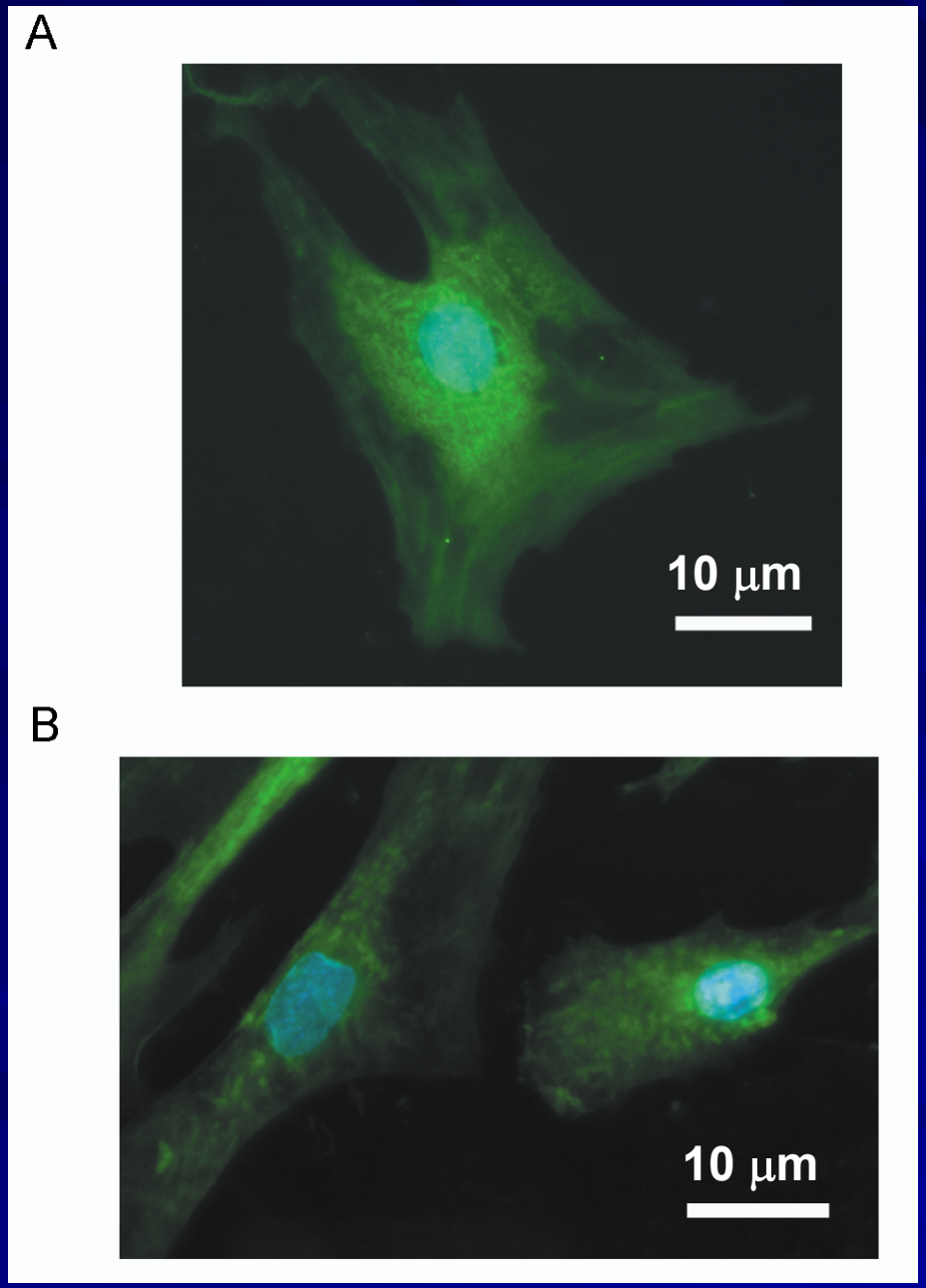
  Miao et al., Life Science, 71:1175-1185, 2002.

  Sandu et al., Diabetes, 49:2178-2189, 2000.

  – **Specific for erectile tissues:**

  Chang et al., Int J Impotence Res 15:53-62, 2003.

  Wingard et al., The Physiologist, 46:237, 2003.



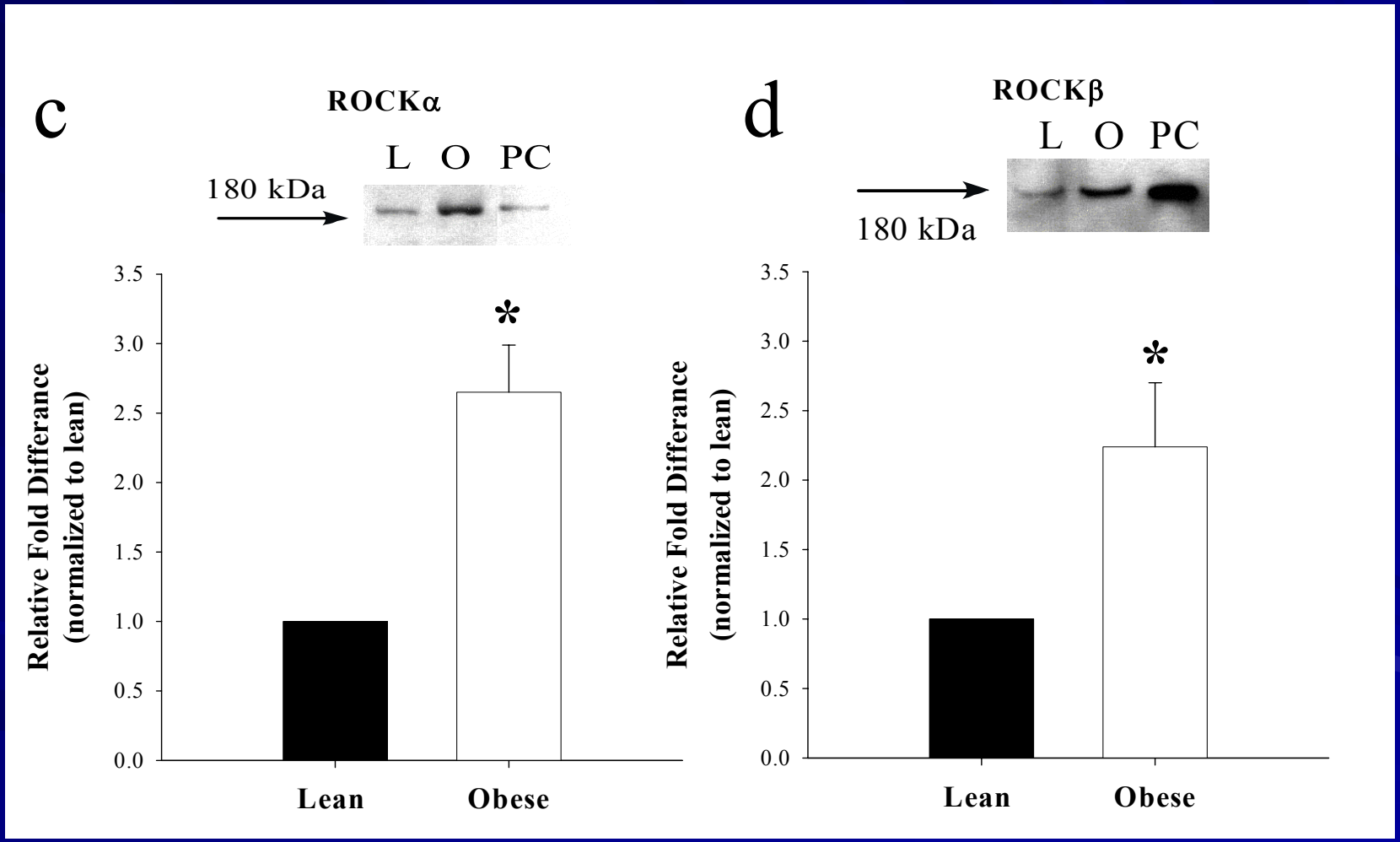
Confocal images of rat cavernosal smooth muscle cells

A: Rho-kinase α

B: RhoA

Green (FITC), blue (DAPI)

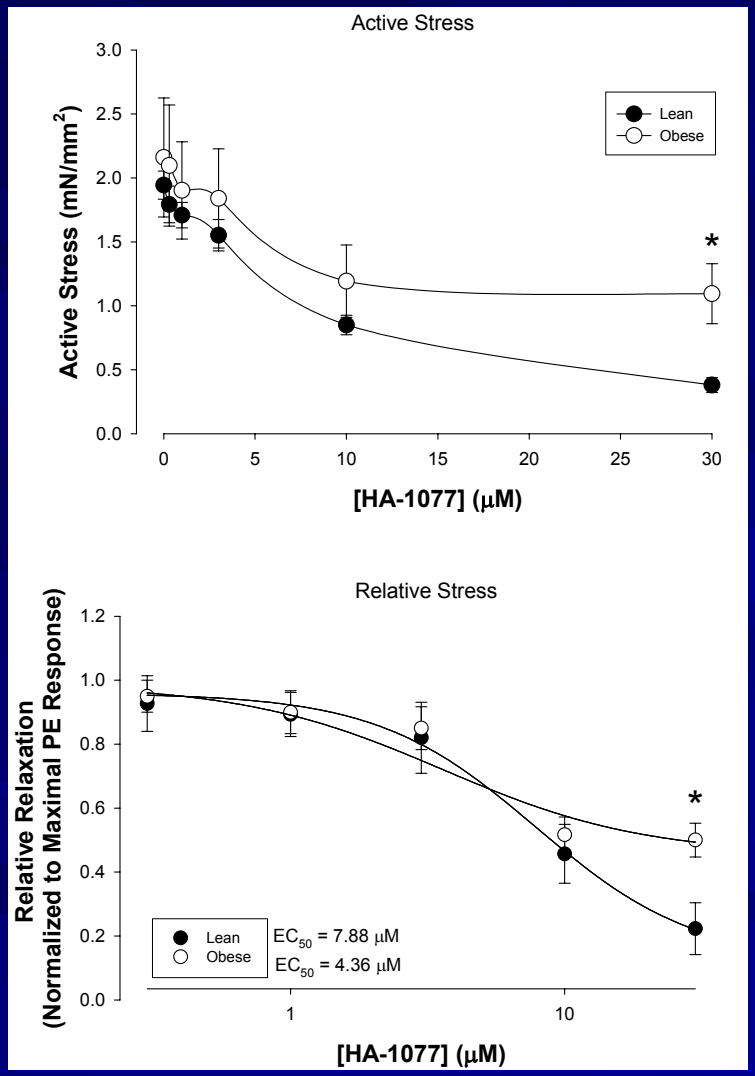
ROCK Expression in Obese-Diabetic Erectile Tissues



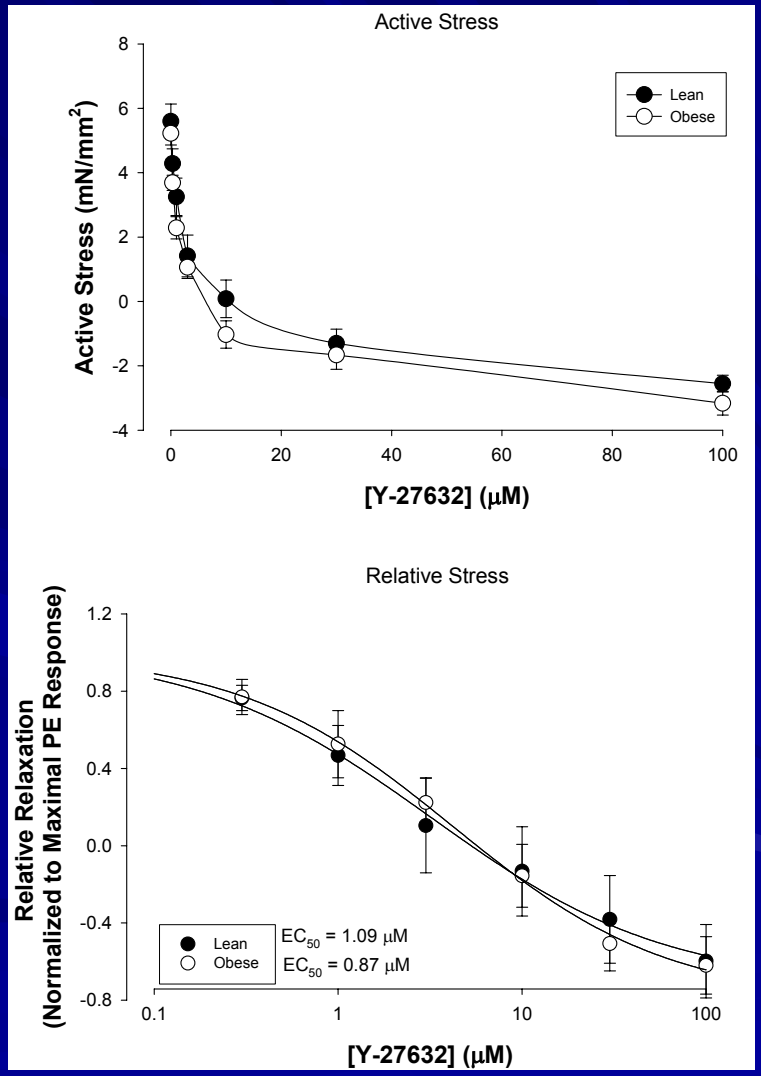
Wingard et al, The Physiologist, 2003

Rho-kinase Inhibition

HA-1077



Y-27632



Wingard et al, Submitted

PKC maintained vasoconstriction in diabetic tissues

- Vascular/urogential tissues:

  Igarashi et al., J. Clin Invest 103:185-195, 1999.

  Qu X, et al., J of Endocrin, 162:207-214, 1999.

  Sandu et al, Diabetes, 49:2178-2189, 2000.

  Miao et al, Life Science, 71:1175-1185, 2002.

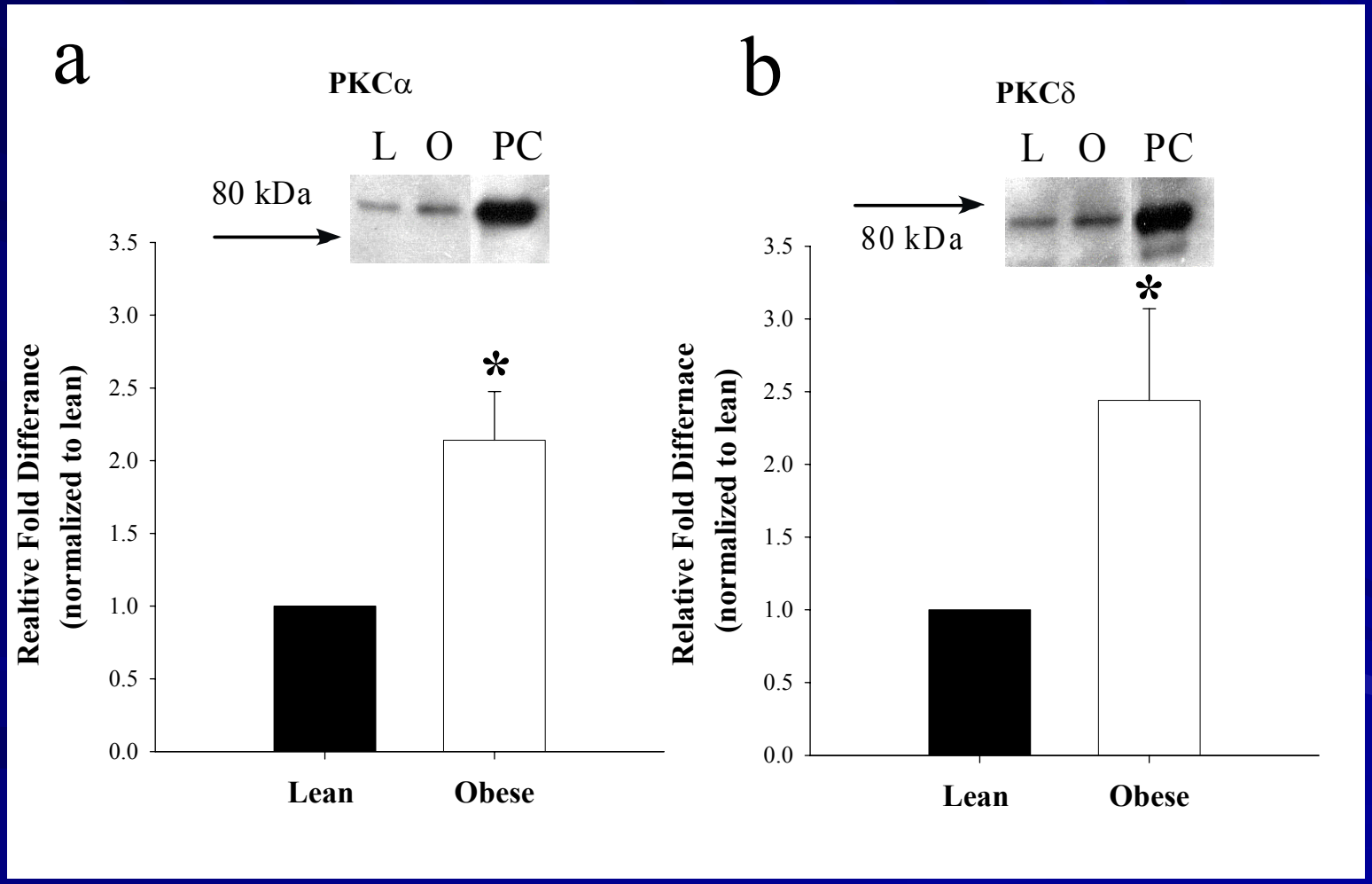
- *Specific for erectile tissues:*

  Nangle et al., Eur J Pharmacol, 475:99-106, 2003.

  Wingard et al , The Physiologist, 46: 237, 2003.

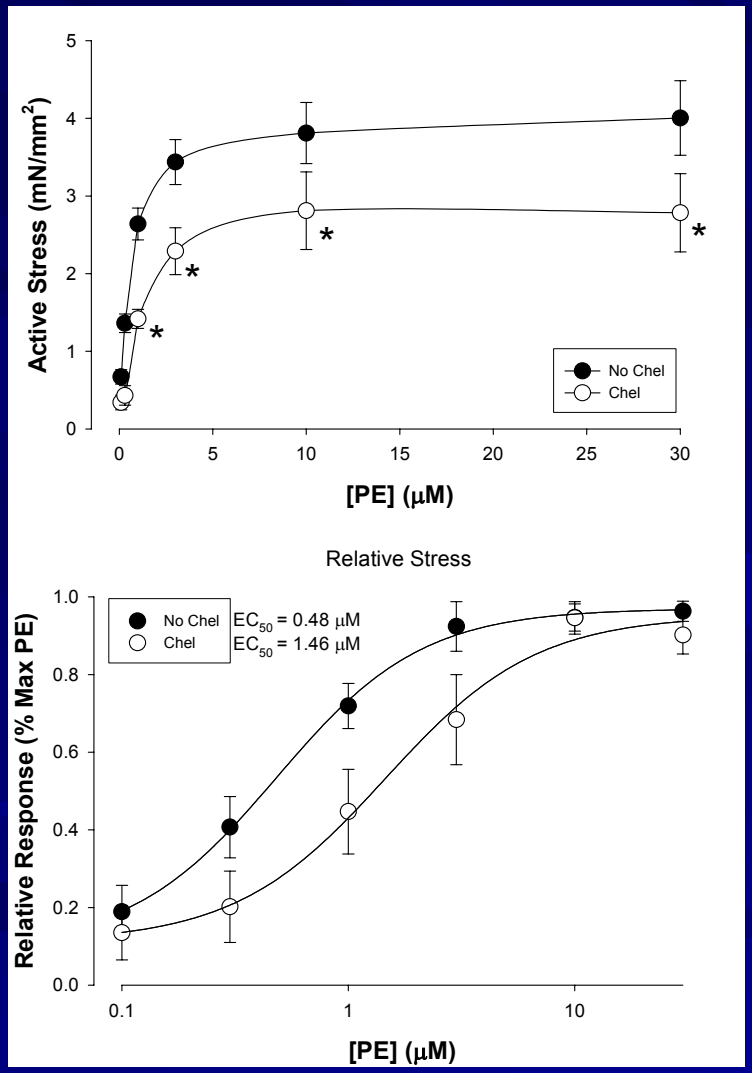
  Husain et al., IJIR, in press, 2003.

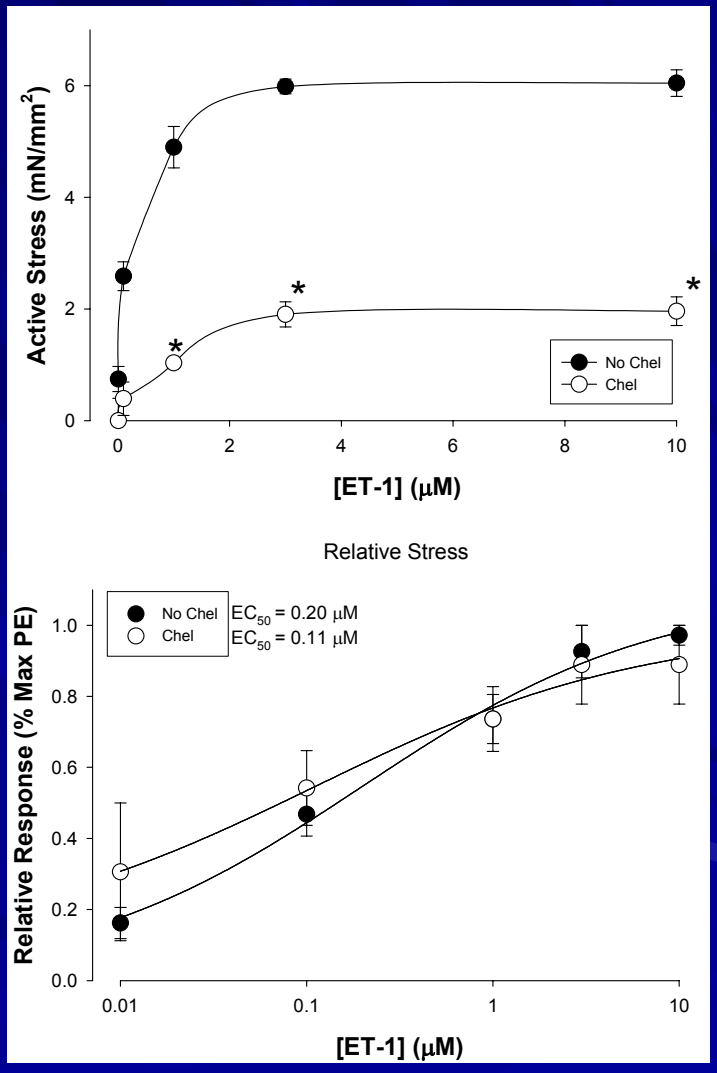
PKC Expression in Obese-Diabetic Erectile Tissues



Wingard et al, Submitted to IJIR

Inhibition of Classical PKCs



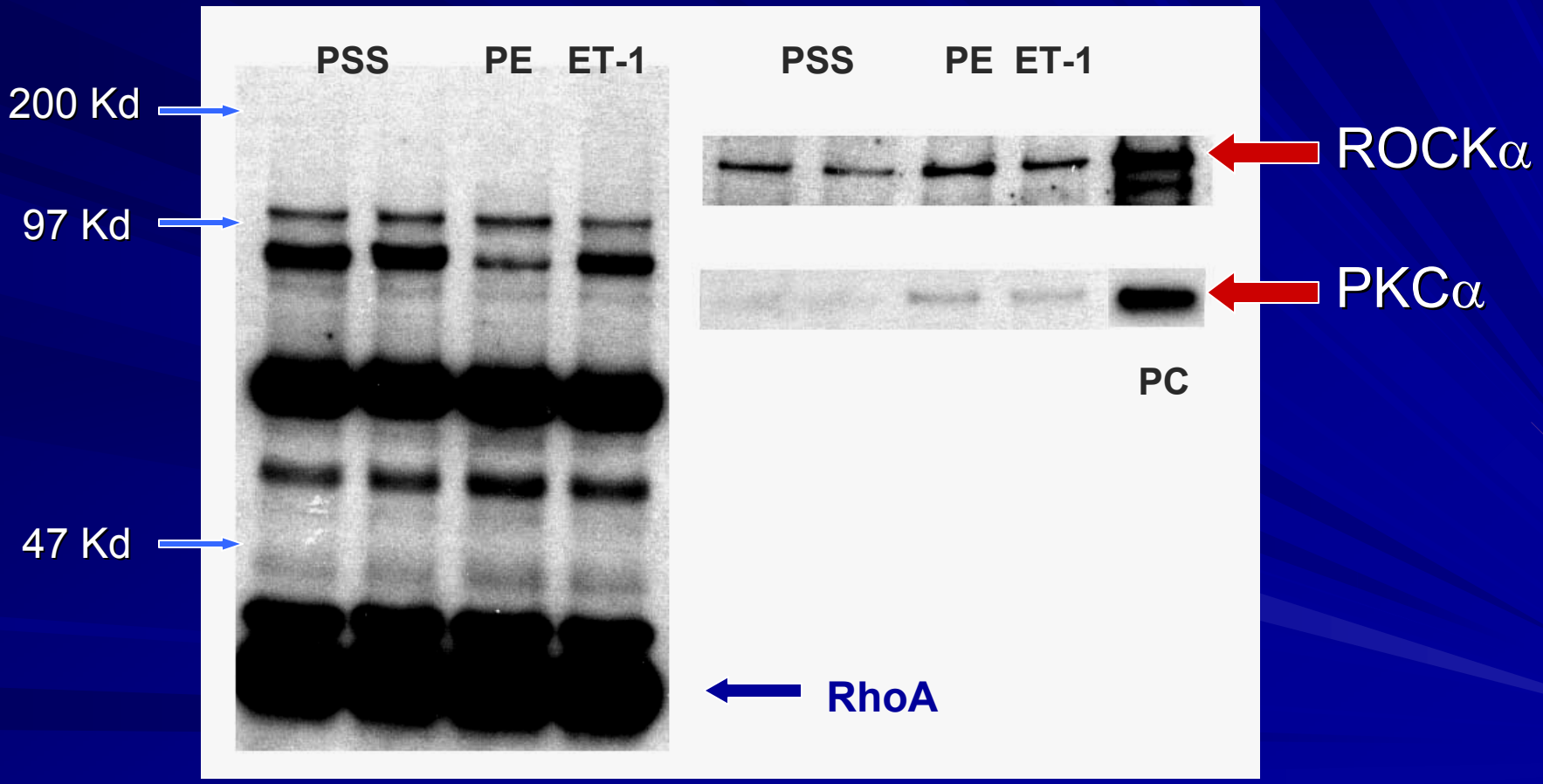


Wingard *et al.*, Physiologist, 46: 237, 2003

PKC RhoA/Rho-kinase Interactions to Regulate Contractile Effects

- Mutual stimulation of Ca^{2+}-sensitization.
  - *Chang, et al., Mol Cell. Biol., 18:49-86-4993, 1998.*
  - *Strassheim, et al., J Biol. Chem.,274:18675-18685, 1999.*
  - *Thieme et al., Inv. Opth and Visual Sci., 41:4240-4246, 2000.*
  - *Slater et al., Biochem 40:4437-4445, 2001*
- Parallel activation of Ca^{2+}-sensitzation processes.
  - *Akopov et al., Am J Physiol., 275: H930-H939, 1998.*
  - *Fu et al., FEBS Lett., 440:183-187, 1998.*
  - *Eto et al., J Biol Chem., 276:29072-29078, 2001.*
  - *Bitar, et al., J App Physiol., 92:41-49, 2002.*

RhoA, ROCKα, and PKCα Association



Conclusions

- Voltage-dependent erectile response in obese-diabetics are lower than lean age-matched animals.
- The constrictors (PE and ET-1) responses of isolated CC from the obese-diabetic animals showed augmented forces.
- Relaxations via ROCK inhibition (HA-1077 or Y-27632) or NO donation (SNP) were not different between lean and obese-diabetic tissues.
- CC tissues from obese-diabetics showed elevated expression of RhoA, ROCK α and β, and PKC α, δ, and ι.
- Agonist stimulation elevated the amount of PKC α and ROCK α co-immunoprecipitated with RhoA.

Continuing Questions

- **Signaling pathway interactions**
  - Do the vasoconstrictors work through a single Ca^{2+}-sensitization process?
  - What are the interactions of the NO/cGMP signaling with Rho/ROCK pathway?
- **Longitudinal changes in diabetic state**
  - What are the consequences of long term glycemic control?
  - What are the anatomical alterations contribution to the reactivity?
- **Clinical consequences**
  - What is the impact of targeting specific elements of the signaling process for treatment of ED?
  - What is the relationship of multi-factorial disorders to erectile function?

Summary and Directions

- Signaling mechanism for the vasoconstrictor pathways in erectile tissue exhibit a complexity similar to that described for other vascular beds.
- Rho/Rock and PKCs appear to play roles in modulation of cavernosal smooth muscle contraction and erection of diabetics.
- Therapeutic targeting of these signaling pathways might be useful in alleviating erectile dysfunction associated with diabetes.