Role of the epithelial Na^+ channel (ENaC) in salt-sensitive hypertension and mechanisms of its regulation by EGF

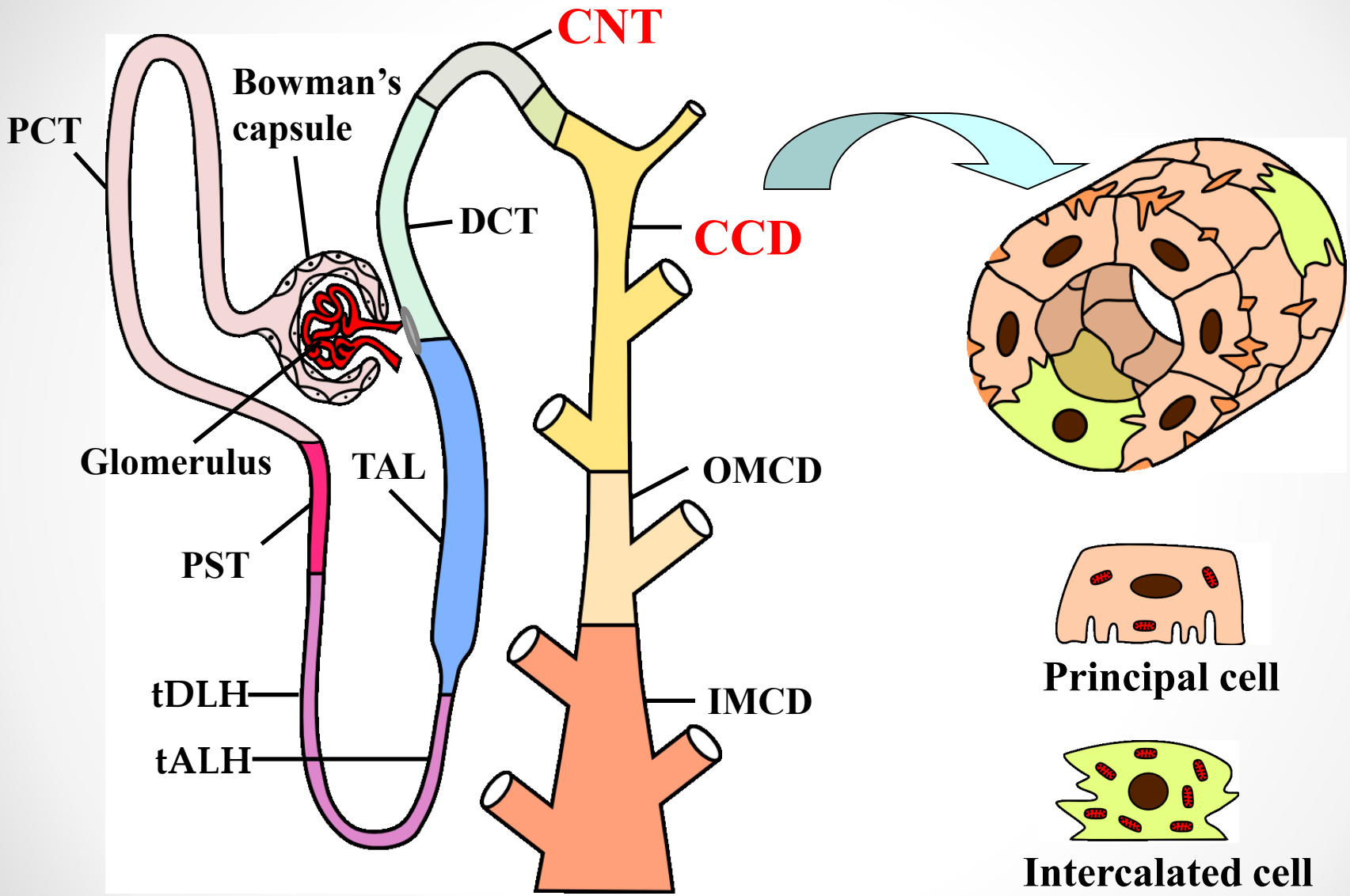
Alexander Staruschenko, PhD

Department of Physiology

MEDICAL COLLEGE OF WISCONSIN

Институт эволюционной физиологии и биохимии им. И. М. Сеченова РАН

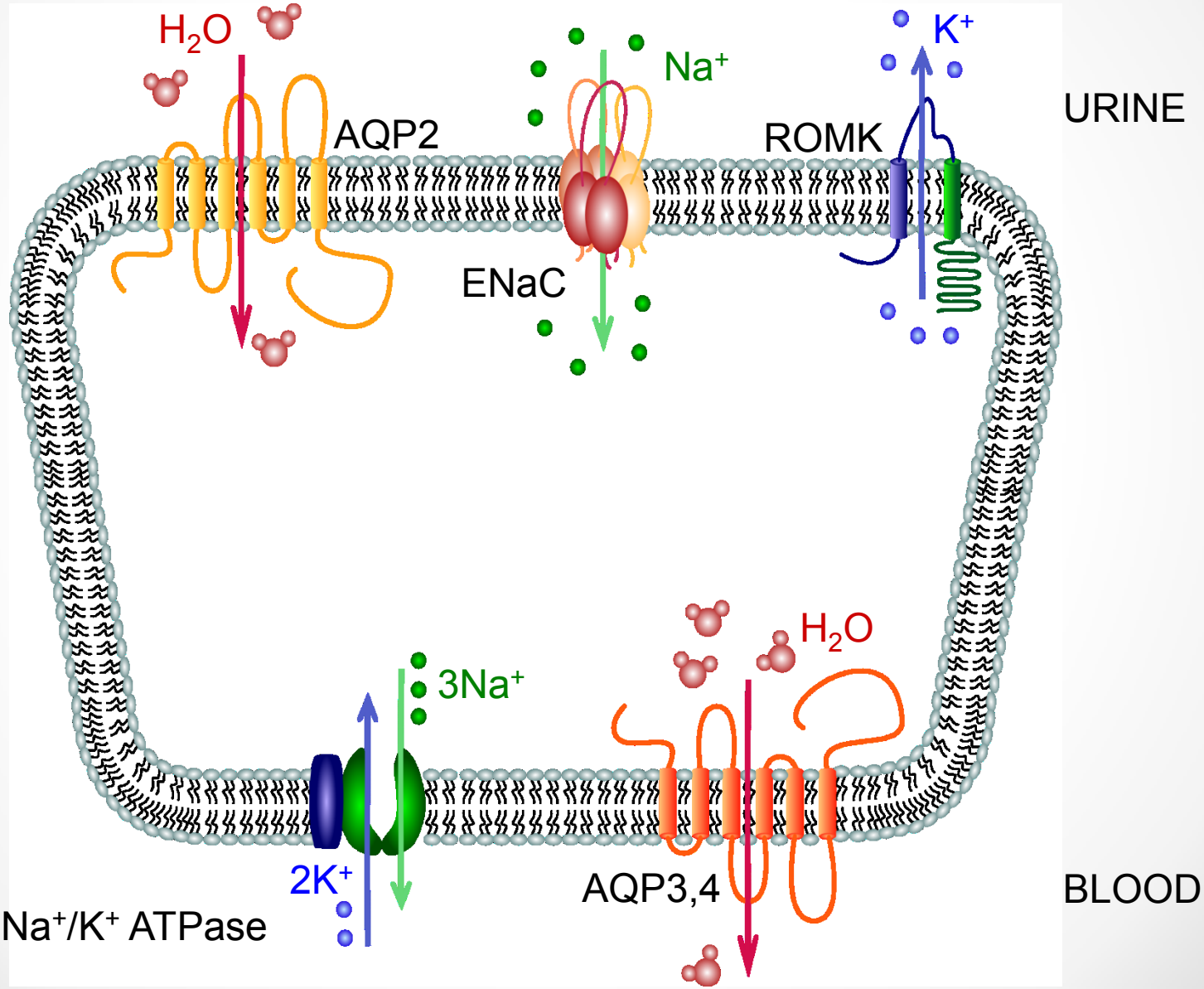
26 декабря 2012 г.



Staruschenko (2012) *Comp. Physiol.* 2(2): 1541-1584.


09/20/2011

The two-membrane model of epithelial transport in principal cells



ENaC regulation

Hormonal factors

Balance of ions

Stretch

Proteolytic cleavage

intracellular signaling

Small GTPase-regulated processes

Arachidonic acid metabolites

PIP pathways

Gene expression

ROS production

Cytoskeletal rearrangements

Ubiquitylation

Phosphorylation

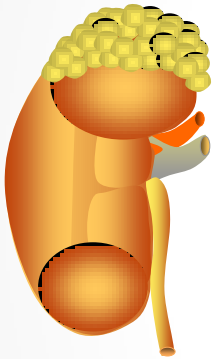
ENaC

- Increased/decreased expression of the channels
- Changes in the open probability
- Altered trafficking

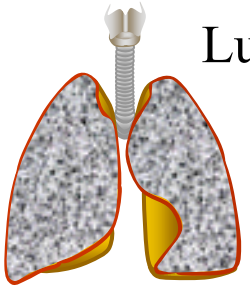
Physiological significance of ENaC

Regulation of the body fluid volume

Kidney



Lungs



Keeps the alveoli dry
Clears the lungs of fluid at birth

sweat and
salivary ducts

Retrieves salt from the sweat

ENaC-associated diseases

Liddle's syndrome
- hypertension
- hypokalemia
- metabolic alcalosis

Pseudohypoaldosteronism type I
- salt wasting
- hyperkalaemia
- unresponsiveness to mineralocorticoids

Cystic fibrosis, polycystic kidney disease etc

- **Role of ENaC in the development of salt-sensitive hypertension in the Dahl Salt-Sensitive (SS) rats**
- **EGF and its related growth factors regulate ENaC**
- **Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins**
- **EGF might regulates ENaC via Rho GDP-dissociation inhibitor RhoGDIα**
- **EGF modulates ENaC-mediated Na^+ transport in the ASDN and participate in the development of salt-sensitive hypertension**

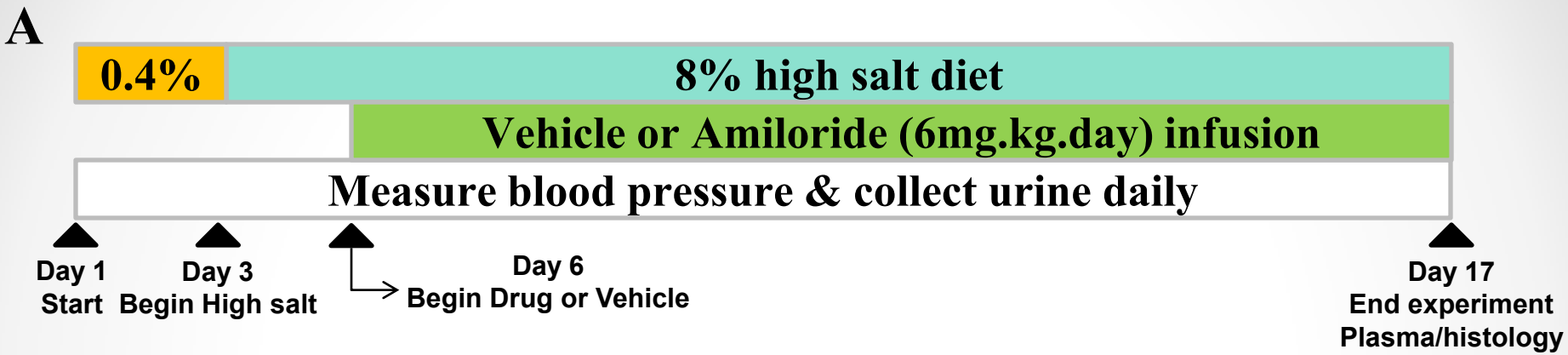
Dahl SS rats

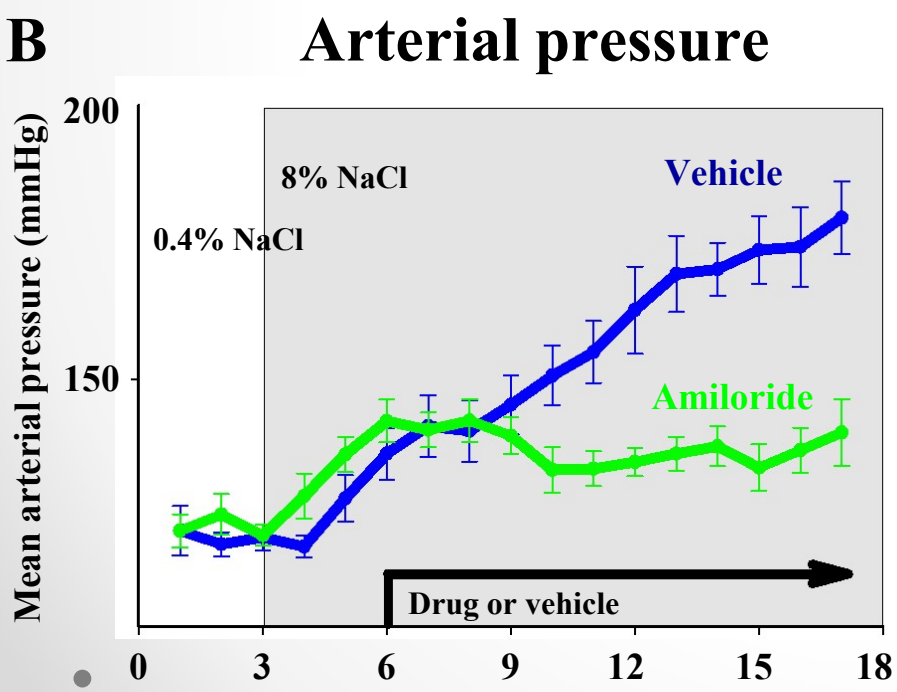
- Blood pressure salt-sensitivity
- Chronic kidney injury
- Reduced renal function
- Low renin hypertension

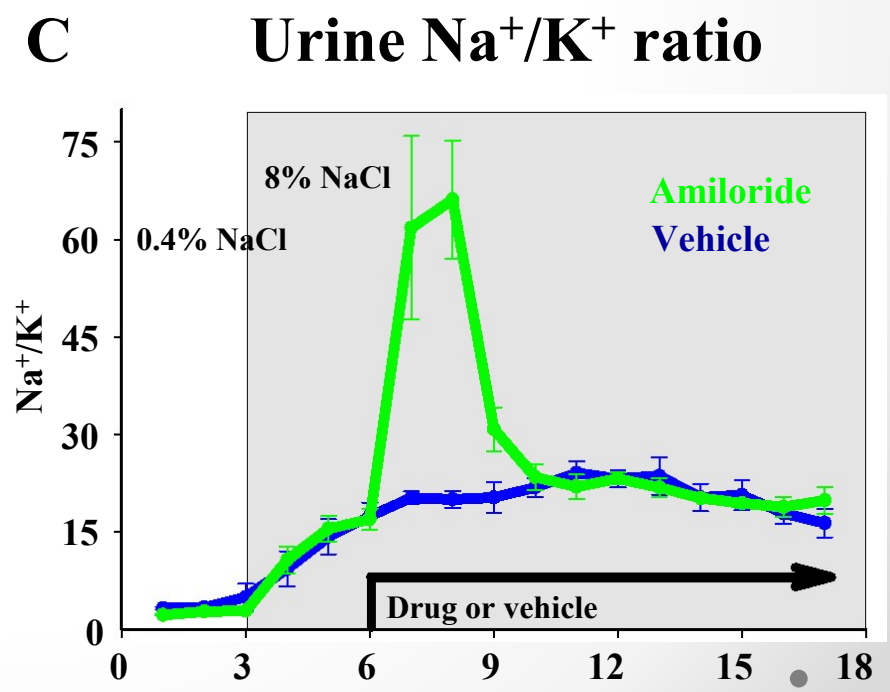
SS.13^{BN}

- consomic rats developed at MCW
- exhibit only 2% allelic differences compared to the SS strain, but salt-induced hypertension and renal injury are greatly attenuated.

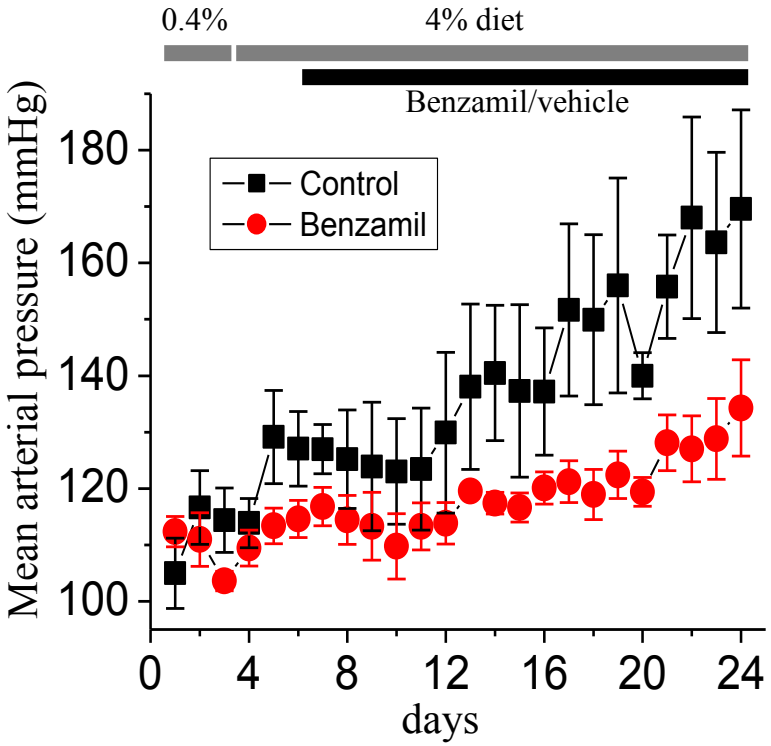
Amiloride inhibits the blood pressure in the SS rats



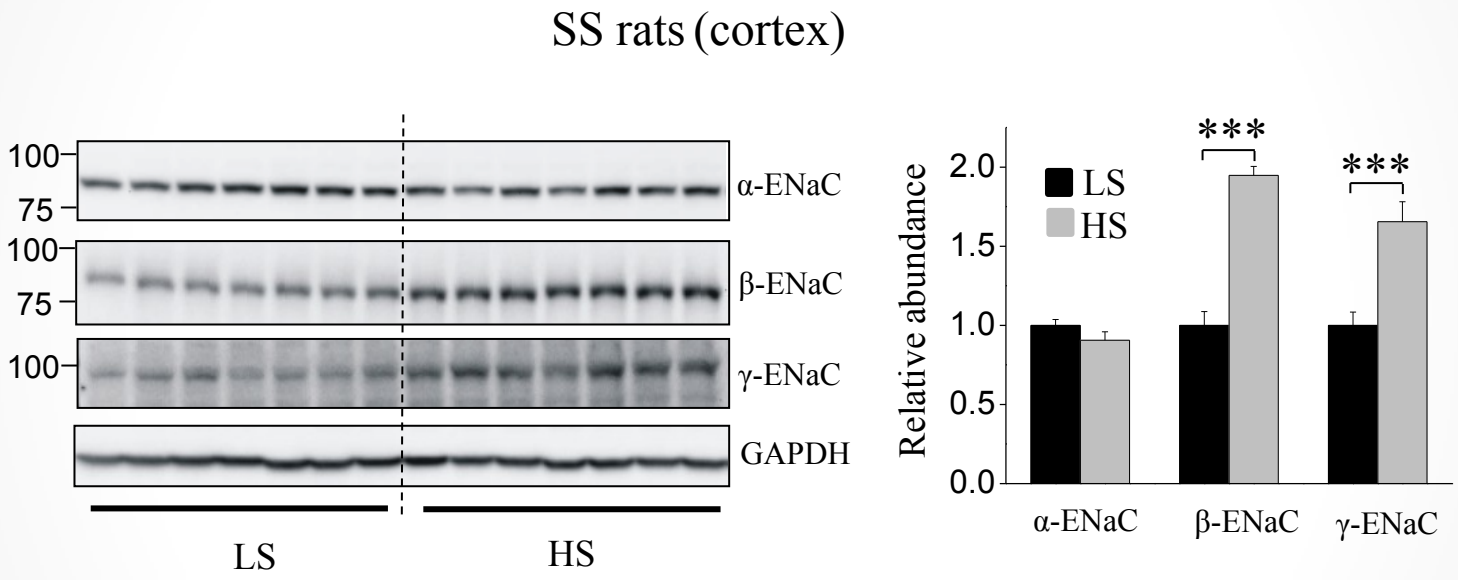




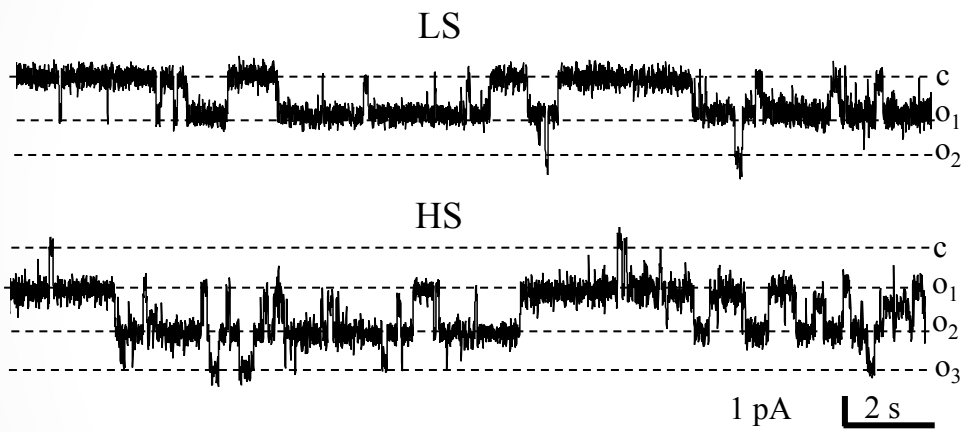
Benzamil (given through drinking water – 15 mg/L) attenuates the development of hypertension in SS rats

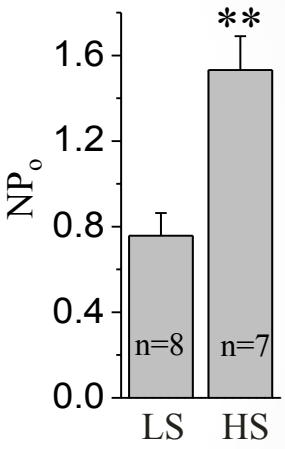


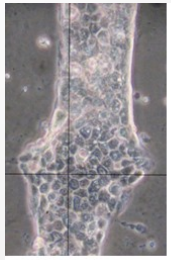
High salt diet increases ENaC expression

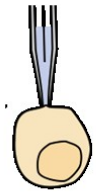


High salt diet increases ENaC activity



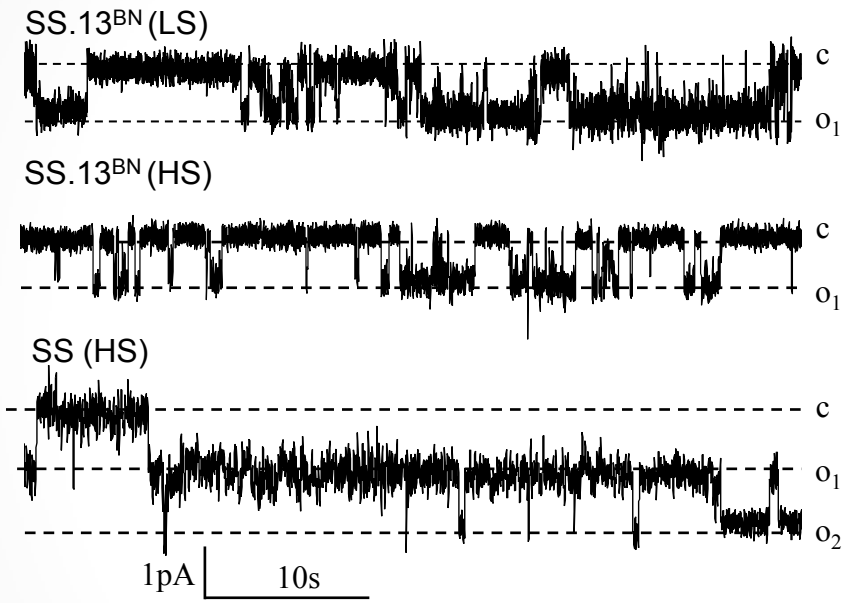


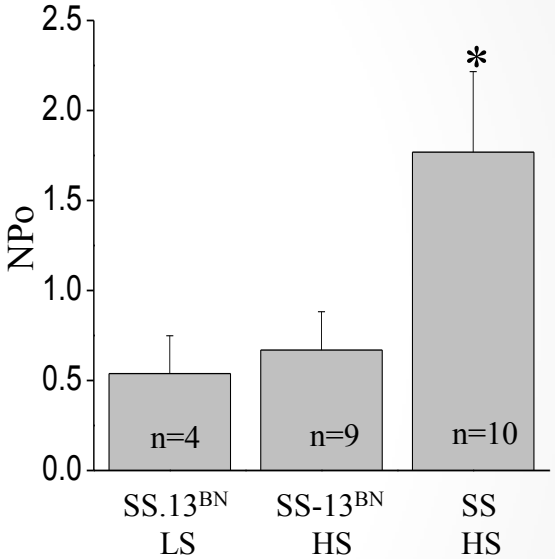


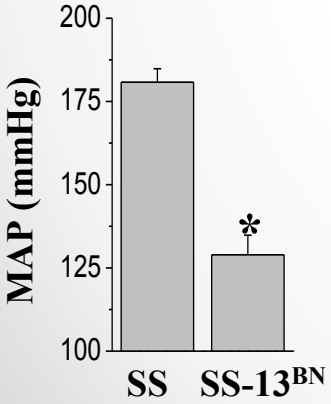


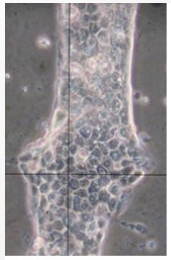
Cell-attached

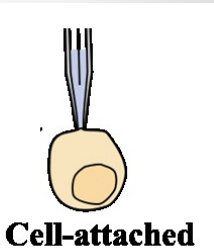
High salt diet increases MAP and ENaC activity in SS rats fed a high salt diet compare to SS.13^{BN}



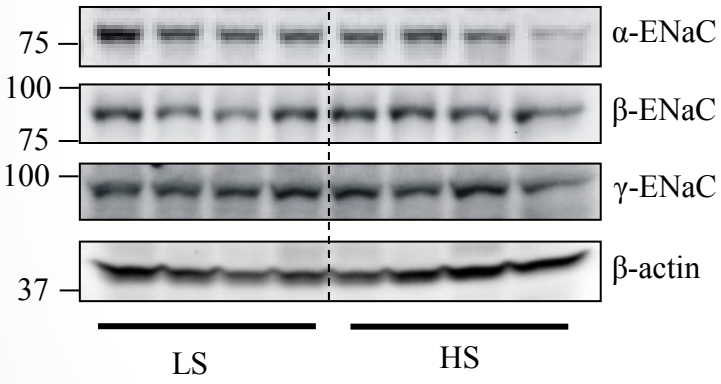


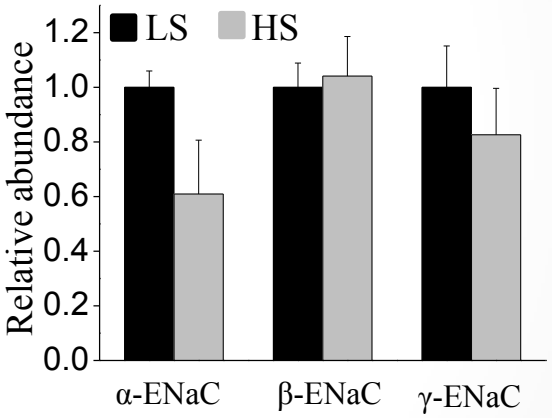






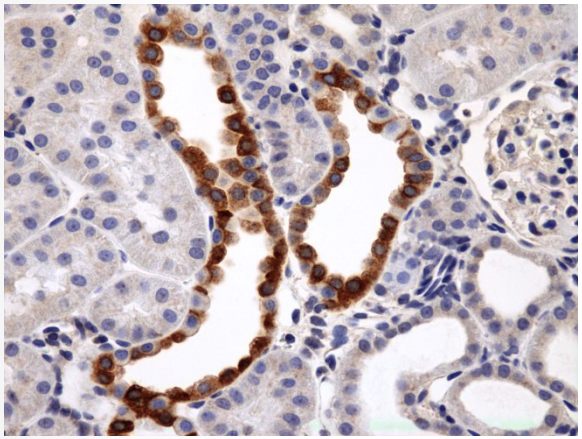
High salt diet does not change ENaC expression in SS.13^{BN} rats



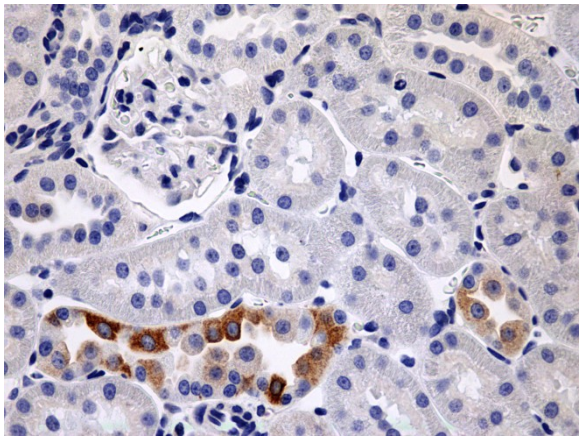


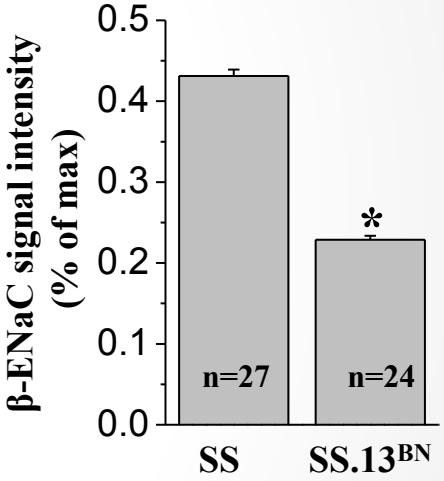
High salt diet increases ENaC abundance in the CCD of SS rats compare to SS.13^{BN}

SS rats

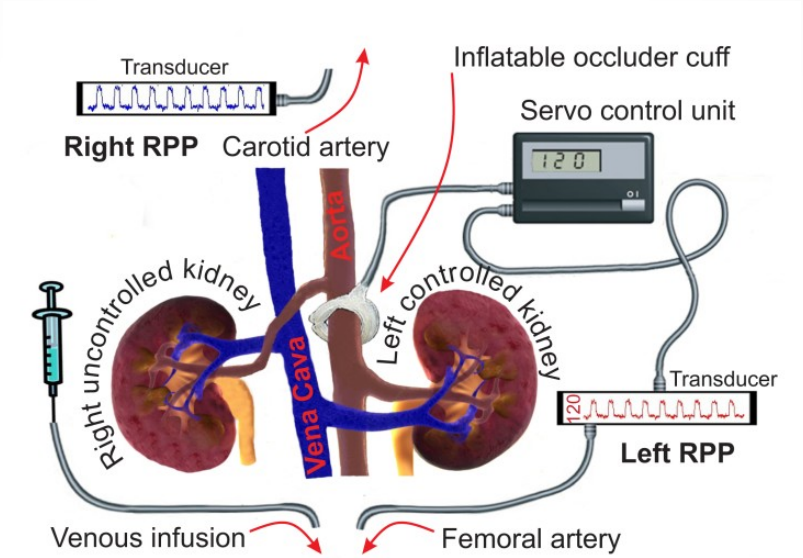


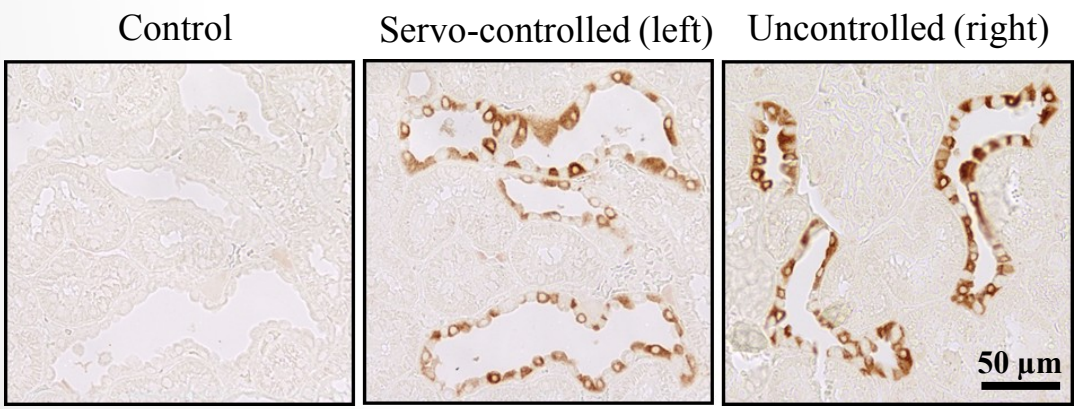
SS.13^{BN}

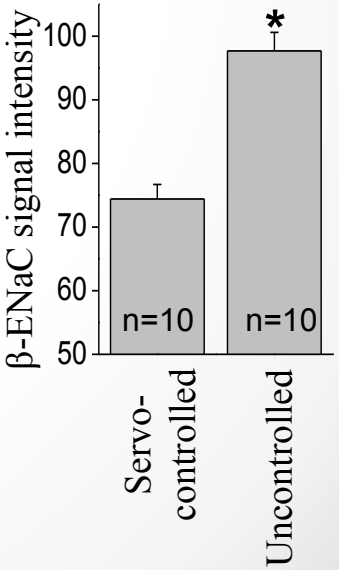




ENaC expression is upregulated in chronic servo-controlled rats





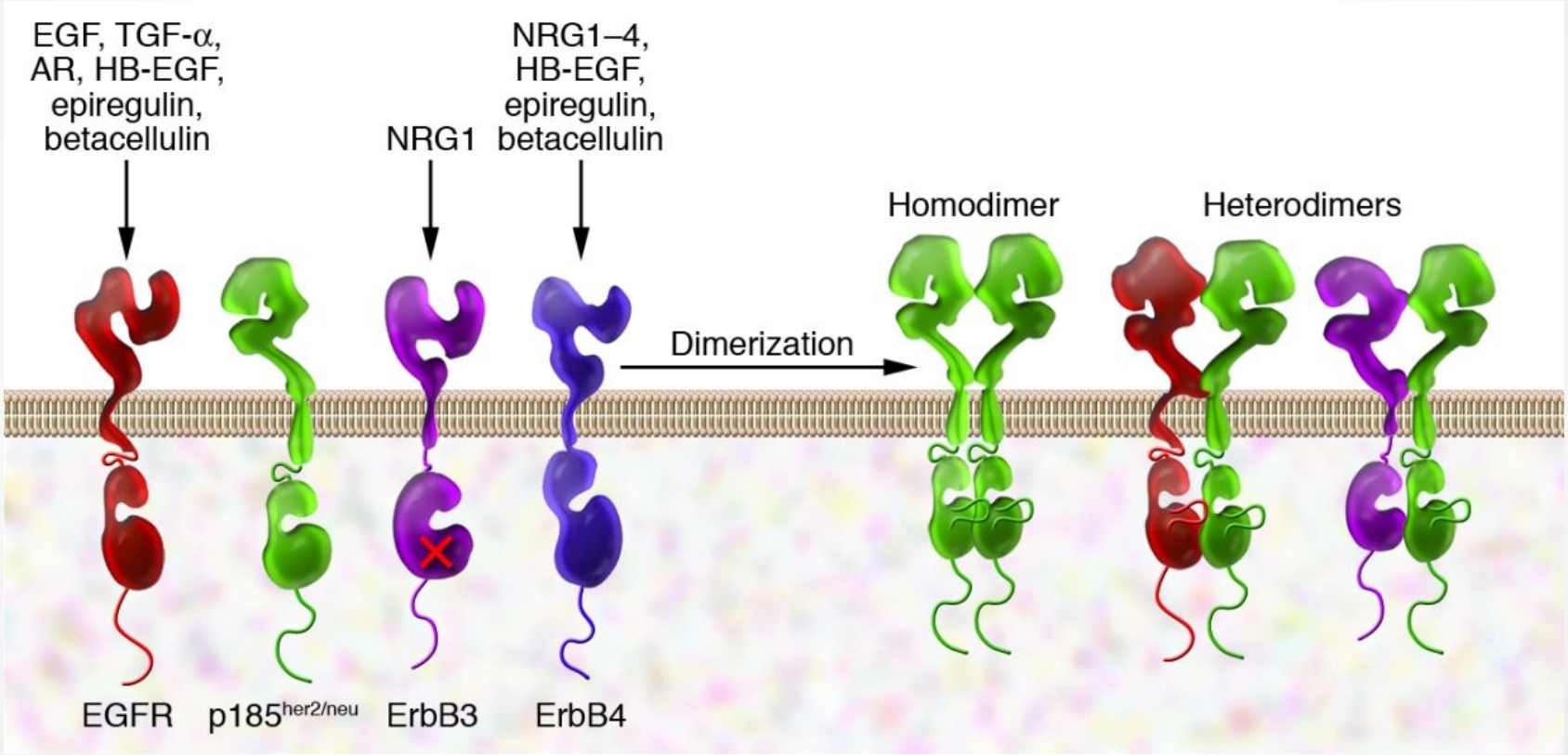


Conclusion:

Dahl salt-sensitive (SS) rats develop severe hypertension on high-salt diet and ENaC contributes to the development of hypertension in the SS rat strain.

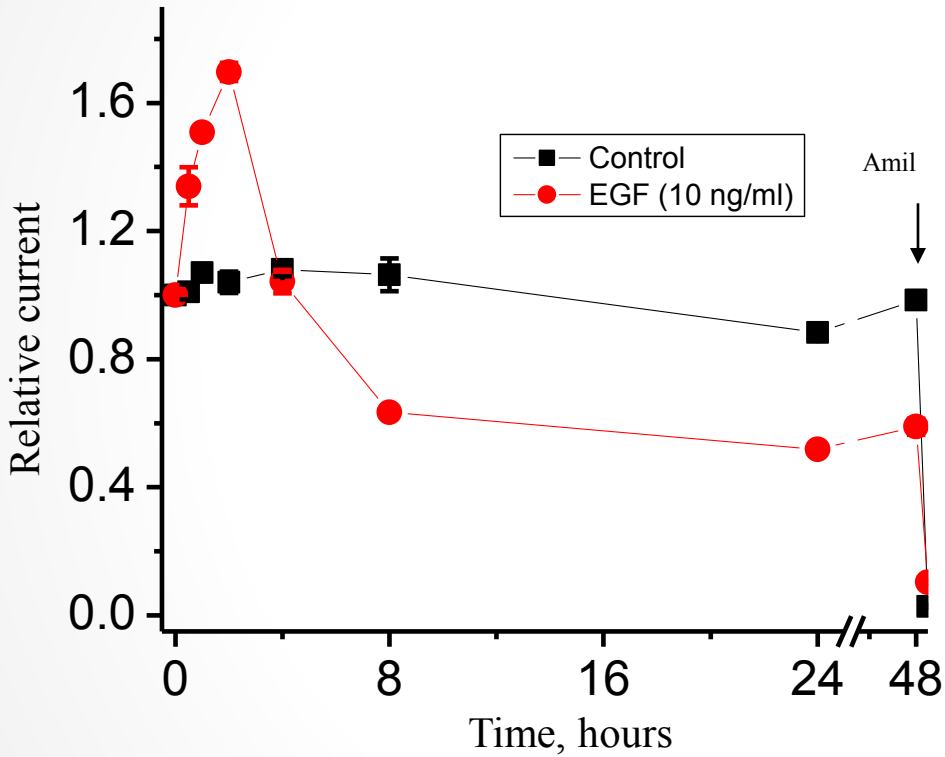
- Role of ENaC in the development of salt-sensitive hypertension in the Dahl Salt-Sensitive (SS) rats
- EGF and its related growth factors regulate ENaC
- Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins
- EGF might regulates ENaC via Rho GDP-dissociation inhibitor RhoGDIα
- EGF modulates ENaC-mediated Na^+ transport in the ASDN and participate in the development of salt-sensitive hypertension

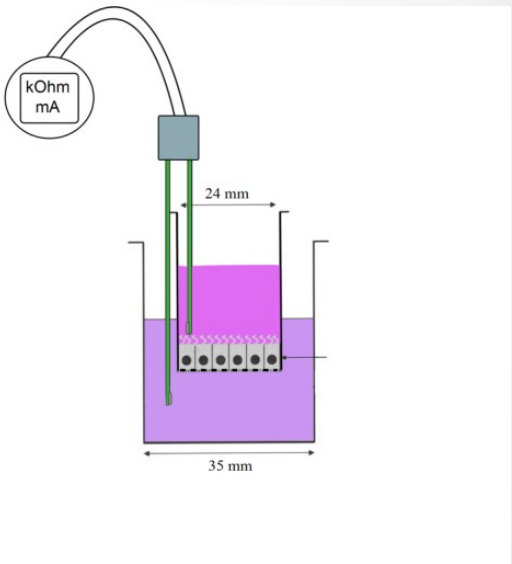
ErbB receptors and their ligands



From: J Clin Invest. 2007; 117:2051–8

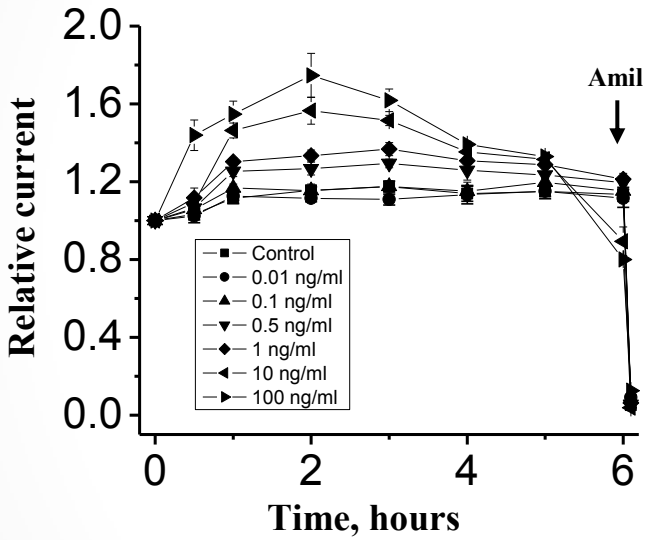
Biphasic effect of EGF on sodium transport in mpkCCD$_{c14}$ cells

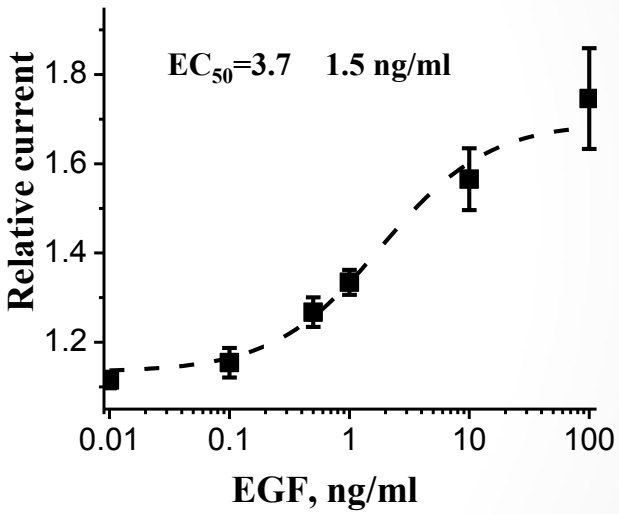


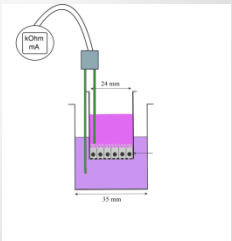


Levchenko et al. (2010) *J Cell Physiol*. 223(1): 252-259.
Liu et al. (2009) *AJP Renal* 296(6): F1417-27.

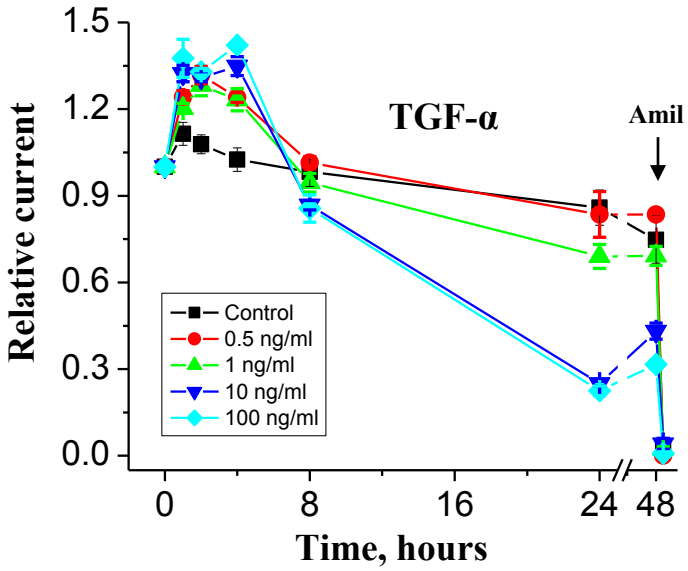
EGF regulates Na^+ transport in a concentration-dependent manner.

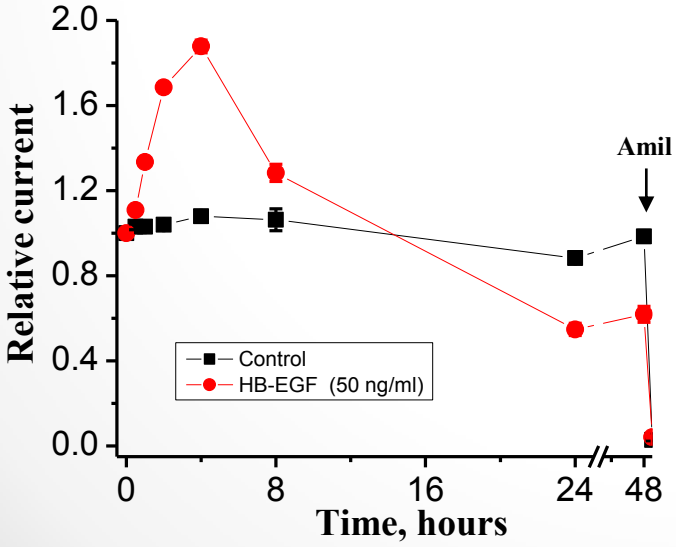


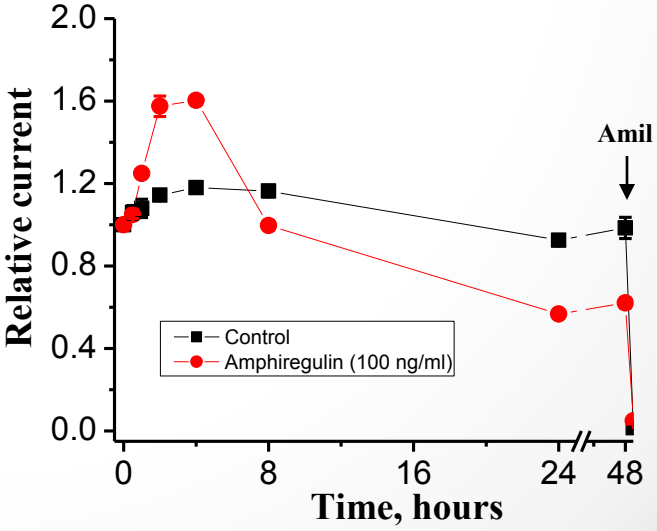


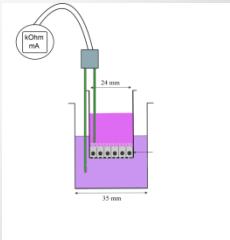


Effect of growth factors in the EGF-family on Na^+ reabsorption across $mpkCCD_{c14}$ monolayer

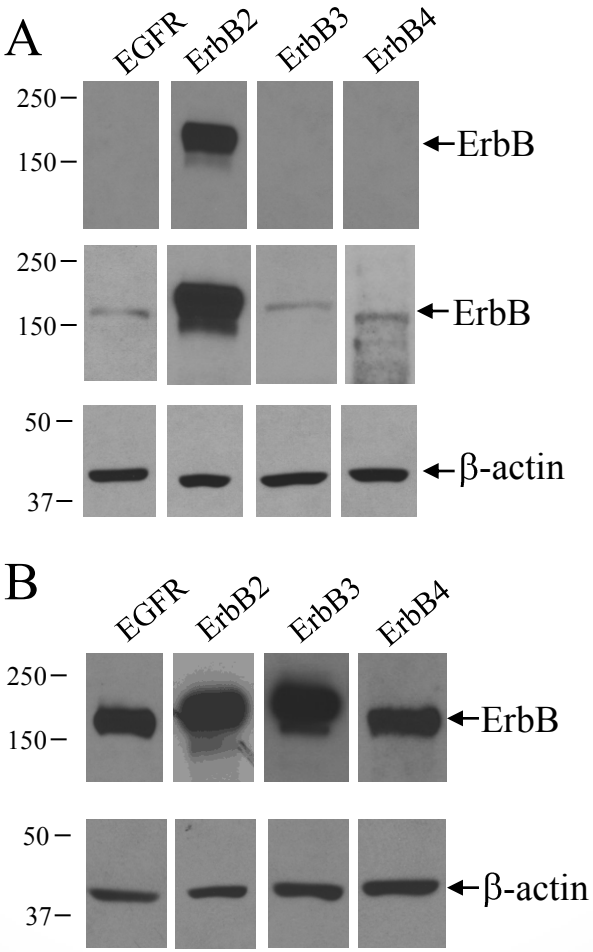






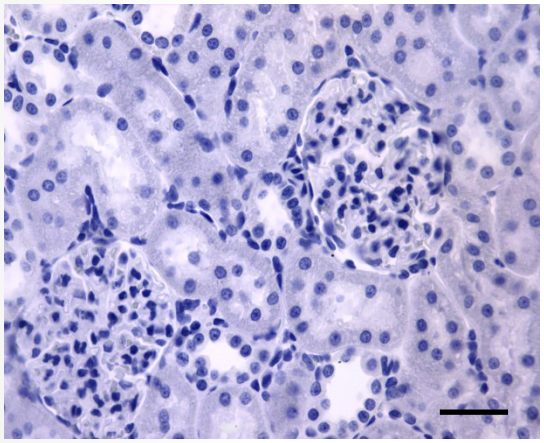


Expression profiles of ErbB receptor family proteins in mpkCCD$_{c14}$ cells analyzed by Western Blot analysis



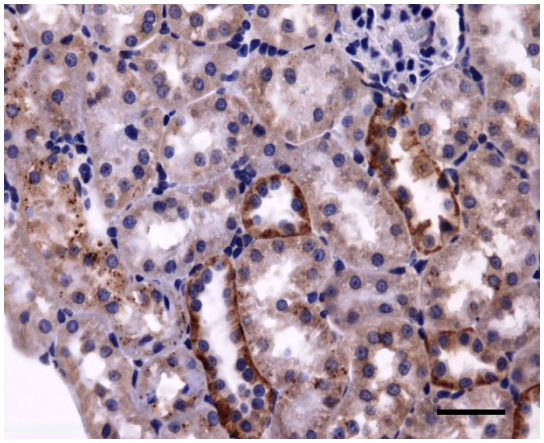
ErbB2 expression in rat kidney tissue

control



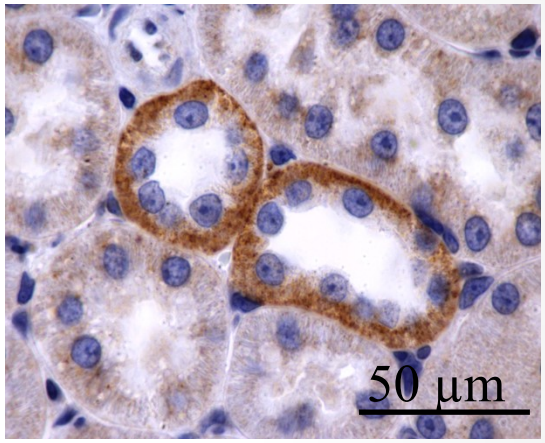
40X

ErbB2



40X

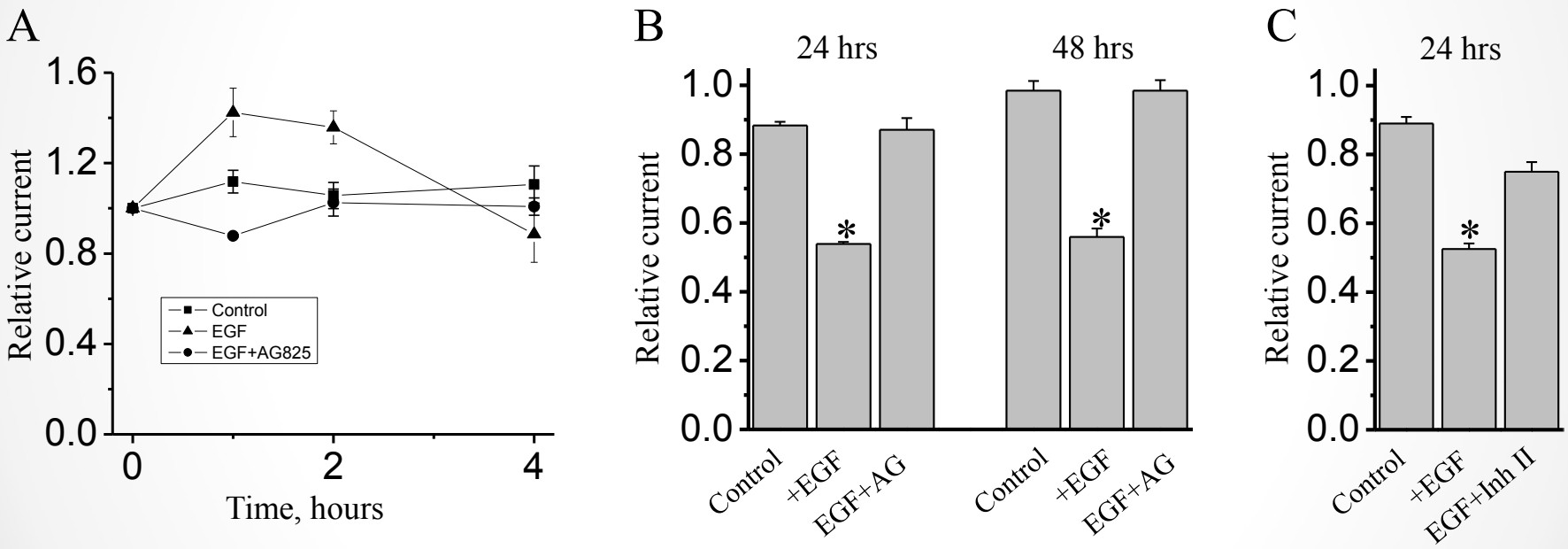
ErbB2

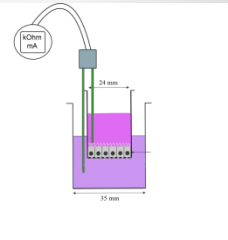


100X

Zheleznova et al. (2011) *BBA - Mol Basis Disease* 408: 242-7.

Effect of ErbB2 inhibitors (tyrphostin AG825 and ErbB2 Inhibitor II) on EGF-induced changes of amiloride-sensitive Na^+ absorption





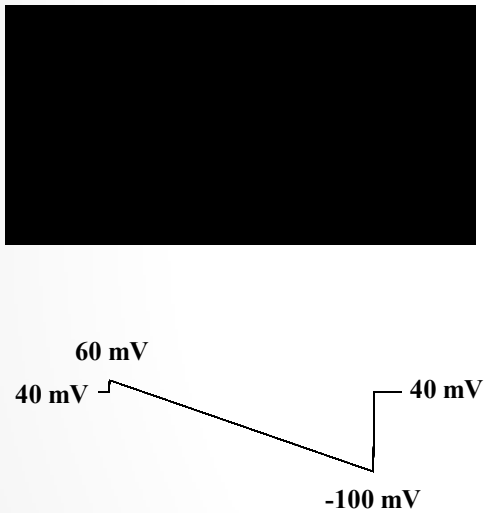
Conclusions:

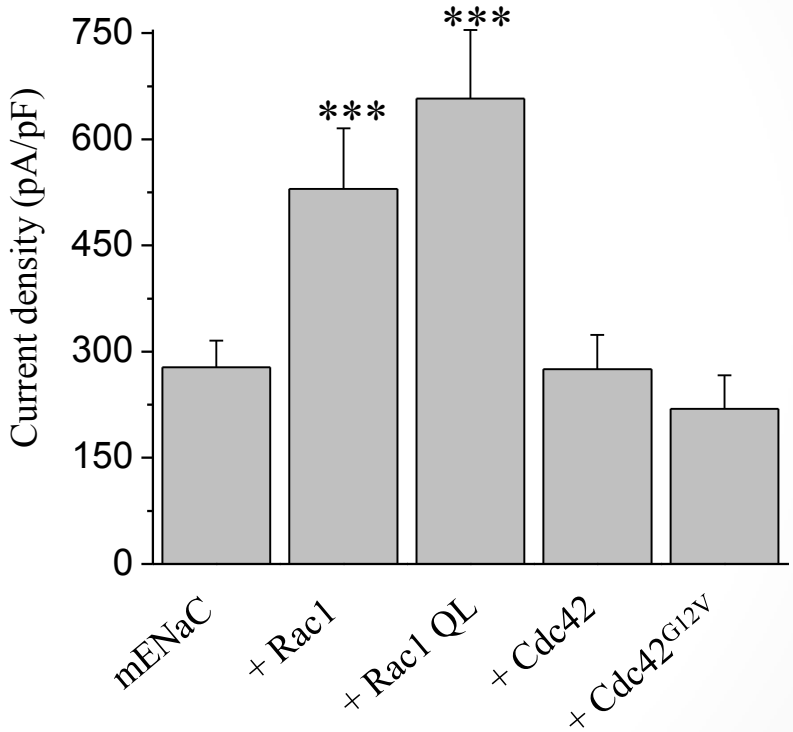
Our data are consistent with the idea that EGF and its related growth factors (TGF-α, HB-EGF and amphiregulin) have a biphasic effect on sodium absorption in the mammalian kidney as represented by $mpkCCD_{c14}$ cells.

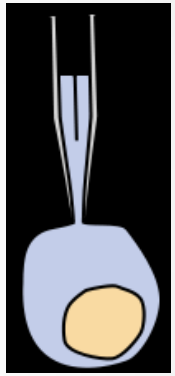
In addition, we show that the EGF effect is mediated via the ErbB2 receptor, most probably via formation of ErbB2/EGFR heterodimers.

- Role of ENaC in the development of salt-sensitive hypertension in the Dahl Salt-Sensitive (SS) rats
- EGF and its related growth factors regulate ENaC
- **Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins**
- EGF might regulates ENaC via Rho GDP-dissociation inhibitor RhoGDIα
- EGF modulates ENaC-mediated Na^+ transport in the ASDN and participate in the development of salt-sensitive hypertension

Rac1 but not Cdc42 enhances ENaC activity overexpressed in CHO cells



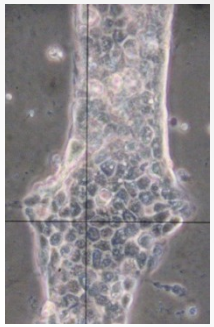


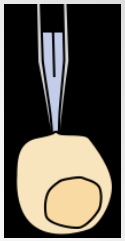


Whole-cell (CHO cells)

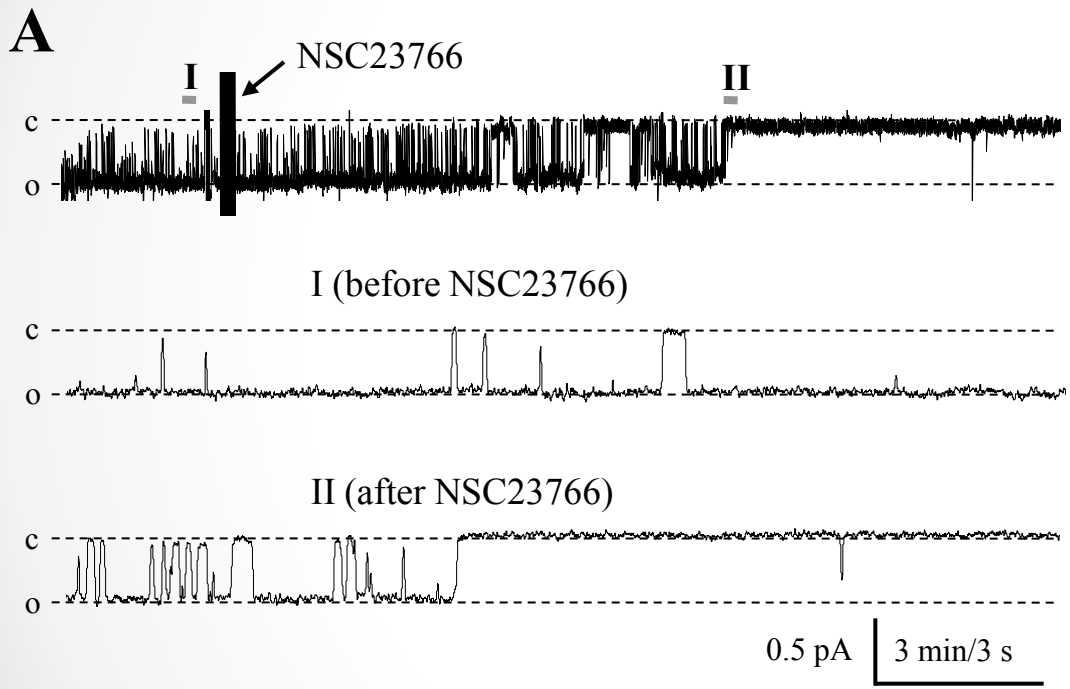
Pavlov et al. 2010 *J Am Soc Nephrol.* 21(5):833-43.

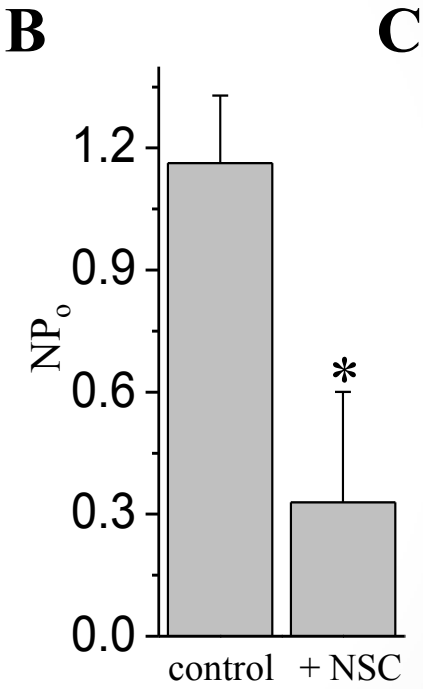
Rac1 is essential for ENaC activity

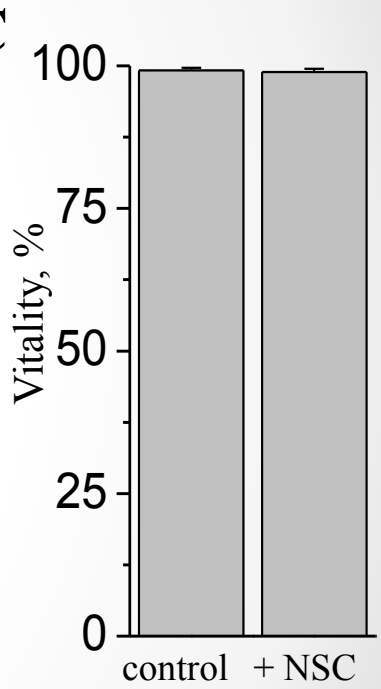




Cell-attached

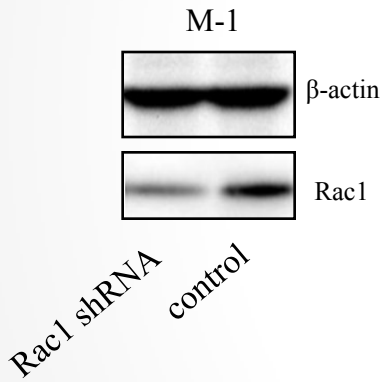


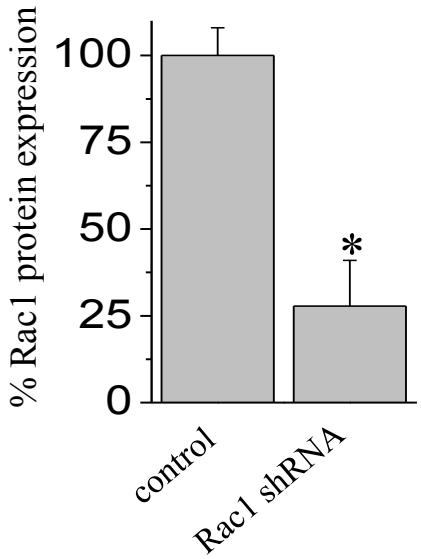


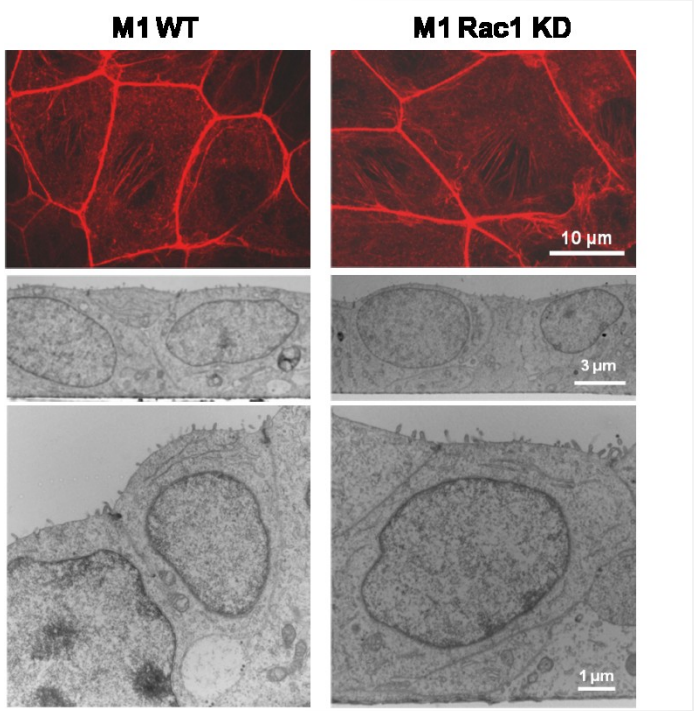


Karpushev et al. (2011) *Hypertension* 57:996-1002.

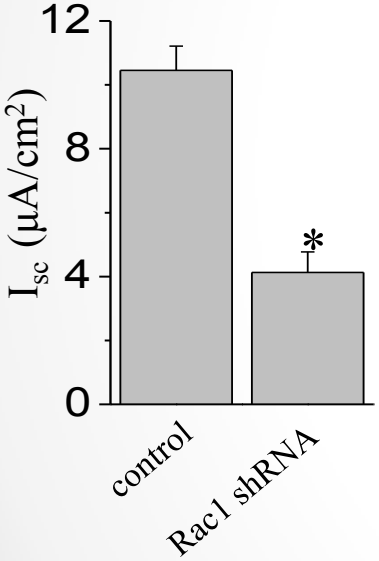
Creation of M-1 stable cell line with lentivirus-mediated knock down of Rac1

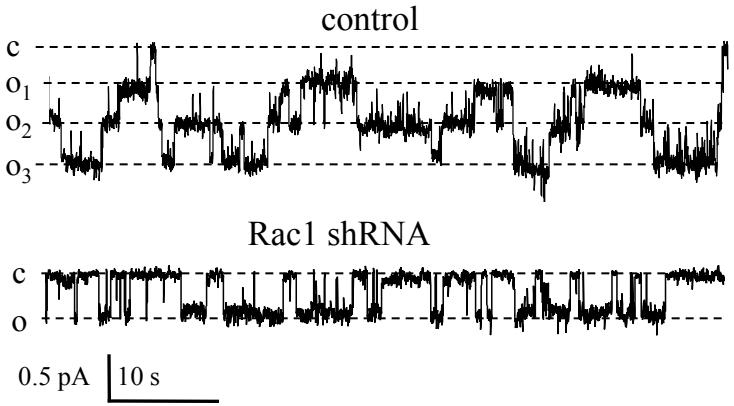


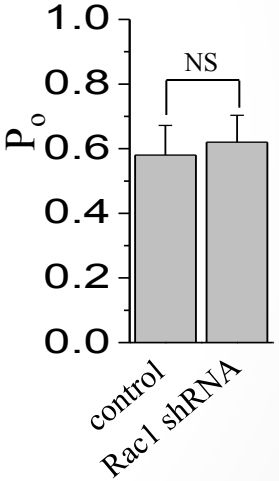


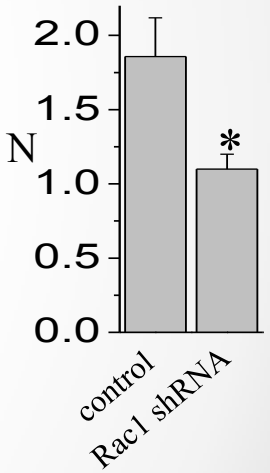


shRNA-mediated silencing of Rac1 inhibit transepithelial Na^+ currents and ENaC activity in M-1 cells

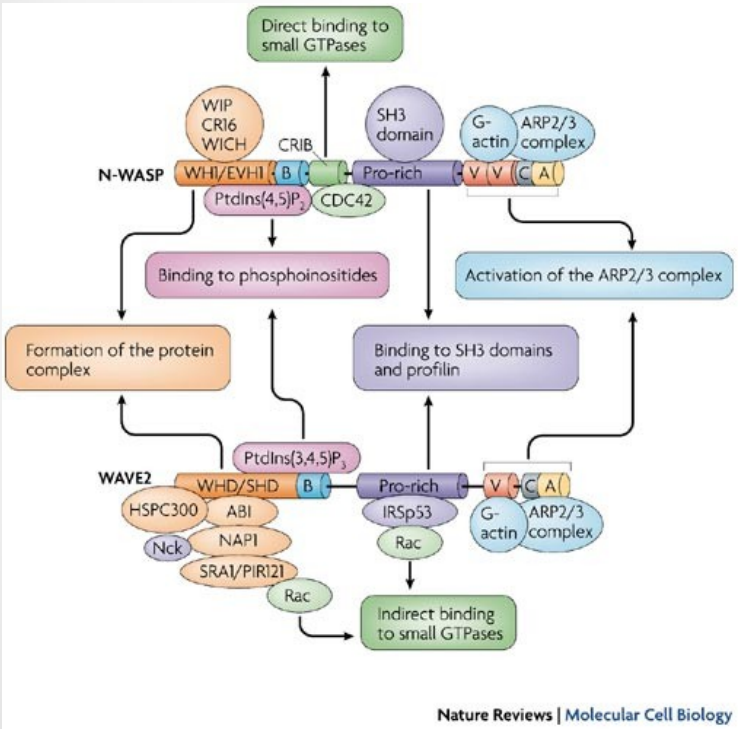




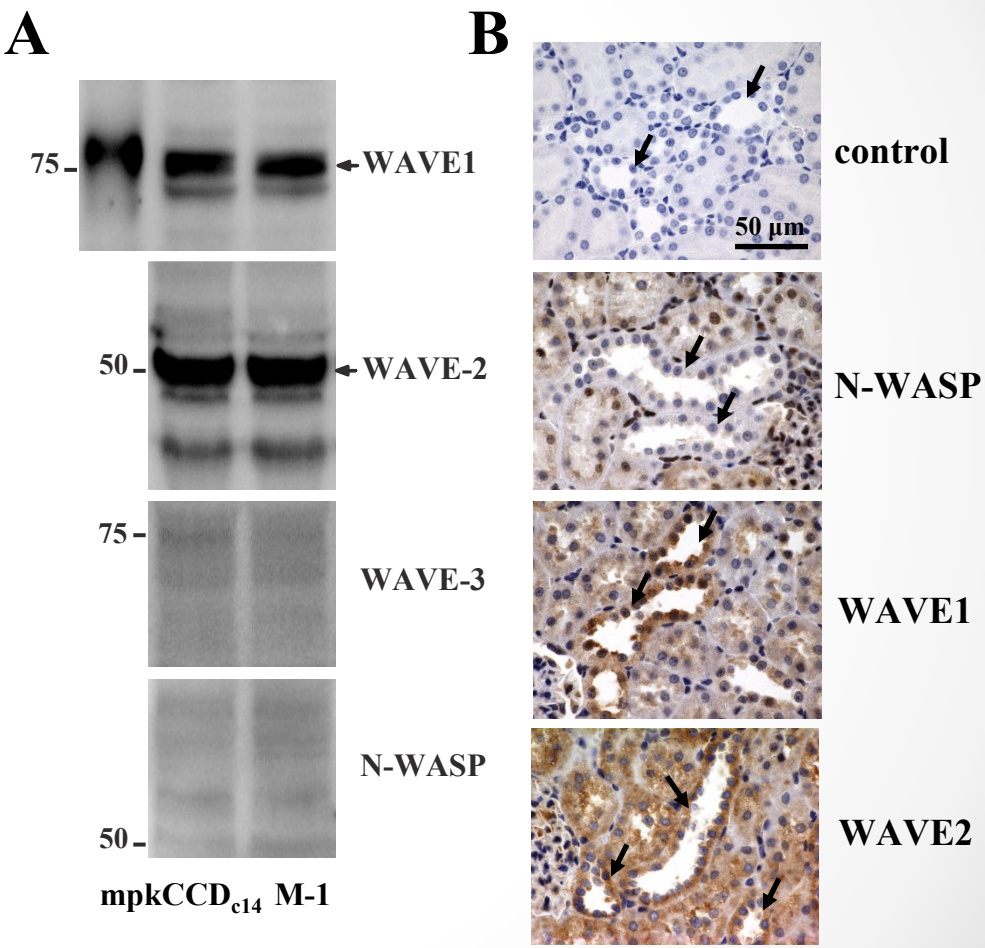




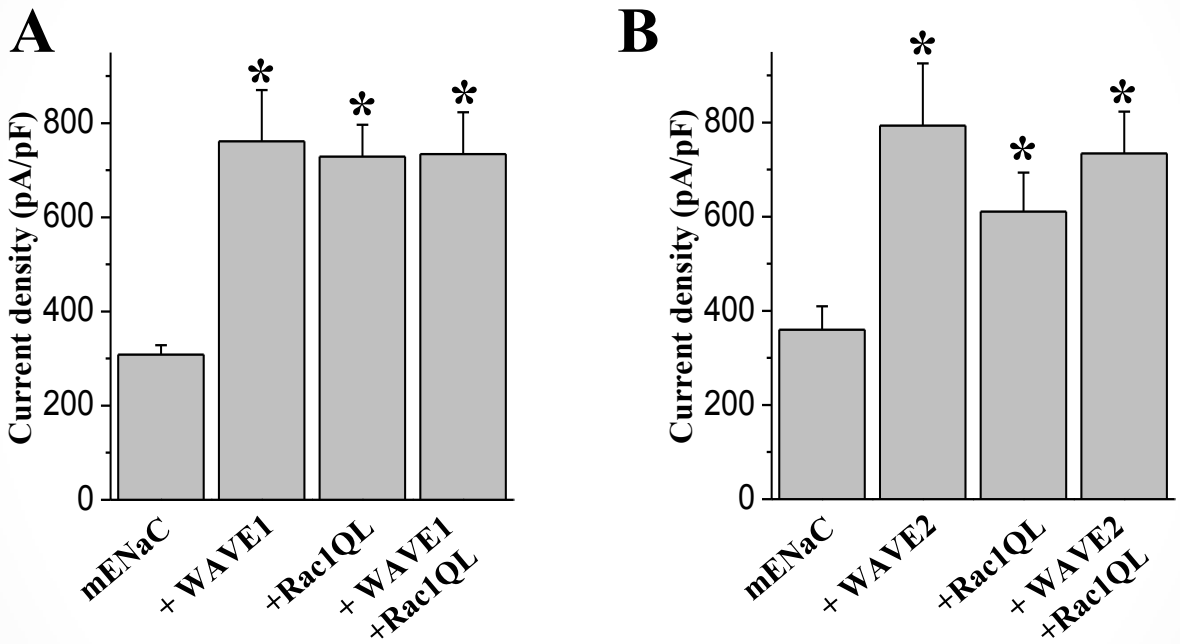
Expression profiles of N-WASP/WAVEs in rat kidney tissue and in epithelial cells

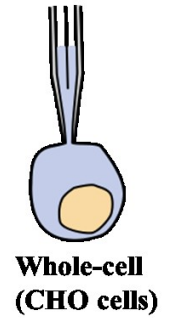


Takenawa and Suetsugu *Nat Rev Mol Cell Biol* **8**, 37–48



Rac1 enhances ENaC activity via WAVE1 or WAVE2





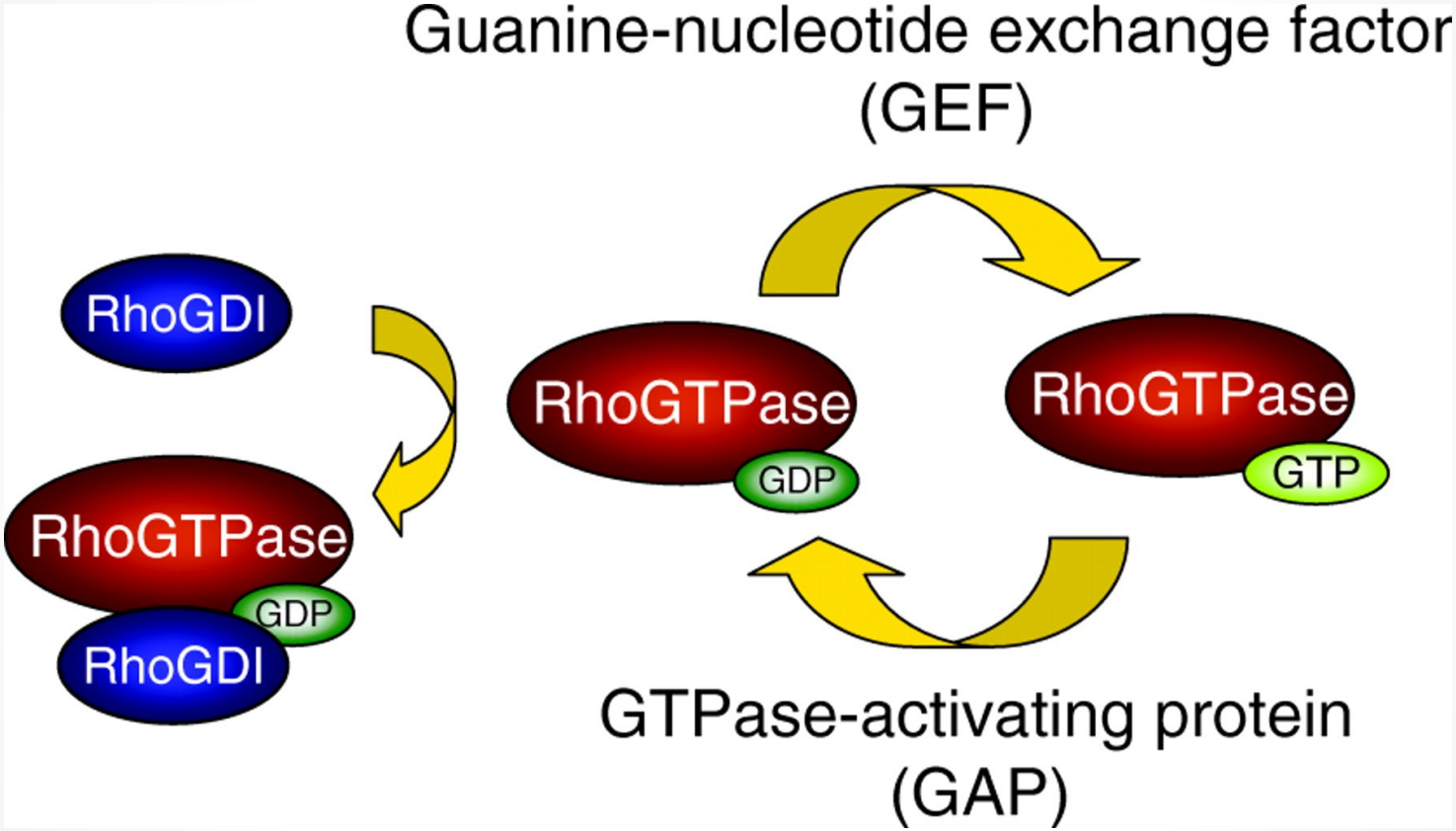
Conclusions:

- Co-expression of Rac1 with ENaC markedly increased channel activity, whereas co-expression of Cdc42 failed to change ENaC activity.
- Inhibition of Rac1-GEF interaction resulted in a decrease of ENaC activity in native principal cells. shRNA-mediated silencing of Rac1 inhibits transepithelial current in M-1 cells via decrease in the number of channels at the plasma membrane.
- Rac1 and WAVE1/2 are parts of the same signaling pathway with respect to activation of ENaC.

- **Thus, our findings suggest that Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins.**

- **Role of ENaC in the development of salt-sensitive hypertension in the Dahl Salt-Sensitive (SS) rats**
- **EGF and its related growth factors regulate ENaC**
- **Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins**
- **EGF might regulates ENaC via Rho GDP-dissociation inhibitor RhoGDIα**
- **EGF modulates ENaC-mediated Na^+ transport in the ASDN and participate in the development of salt-sensitive hypertension**

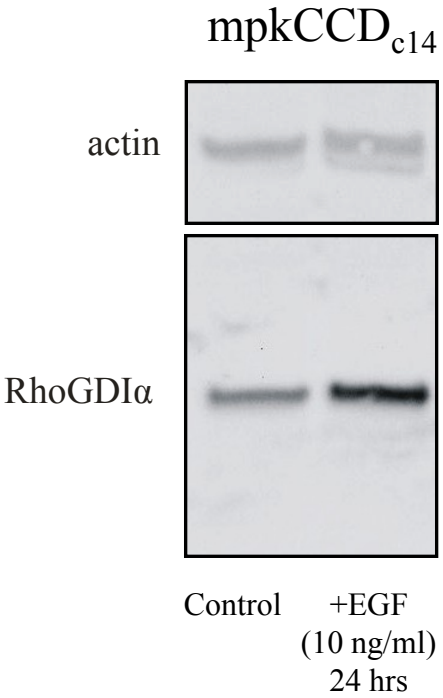
Small GTPases cycle between a GDP- and GTP-bound state



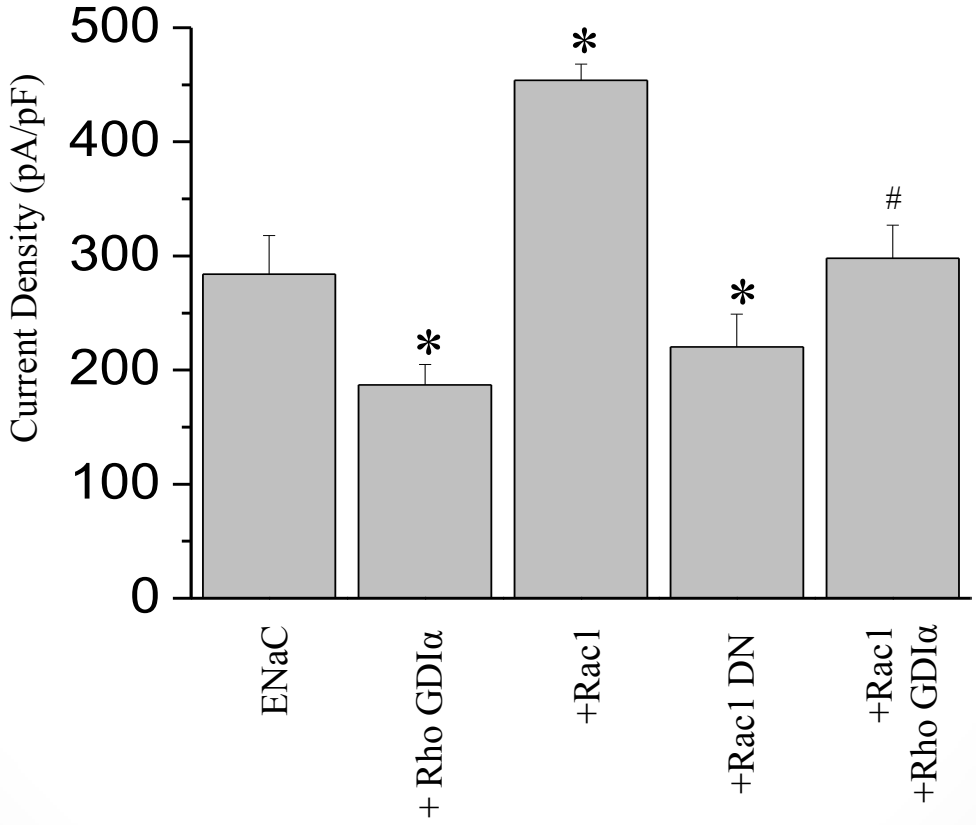
Nethe M , Hordijk P L J Cell Sci 2010;123:4011-4018

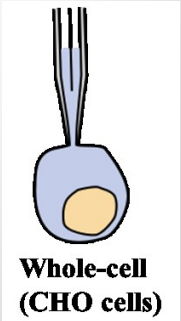
Journal of Cell Science
jcs.biologists.org

EGF upregulates RhoGDIα in mpkCCD$_{c14}$ cells

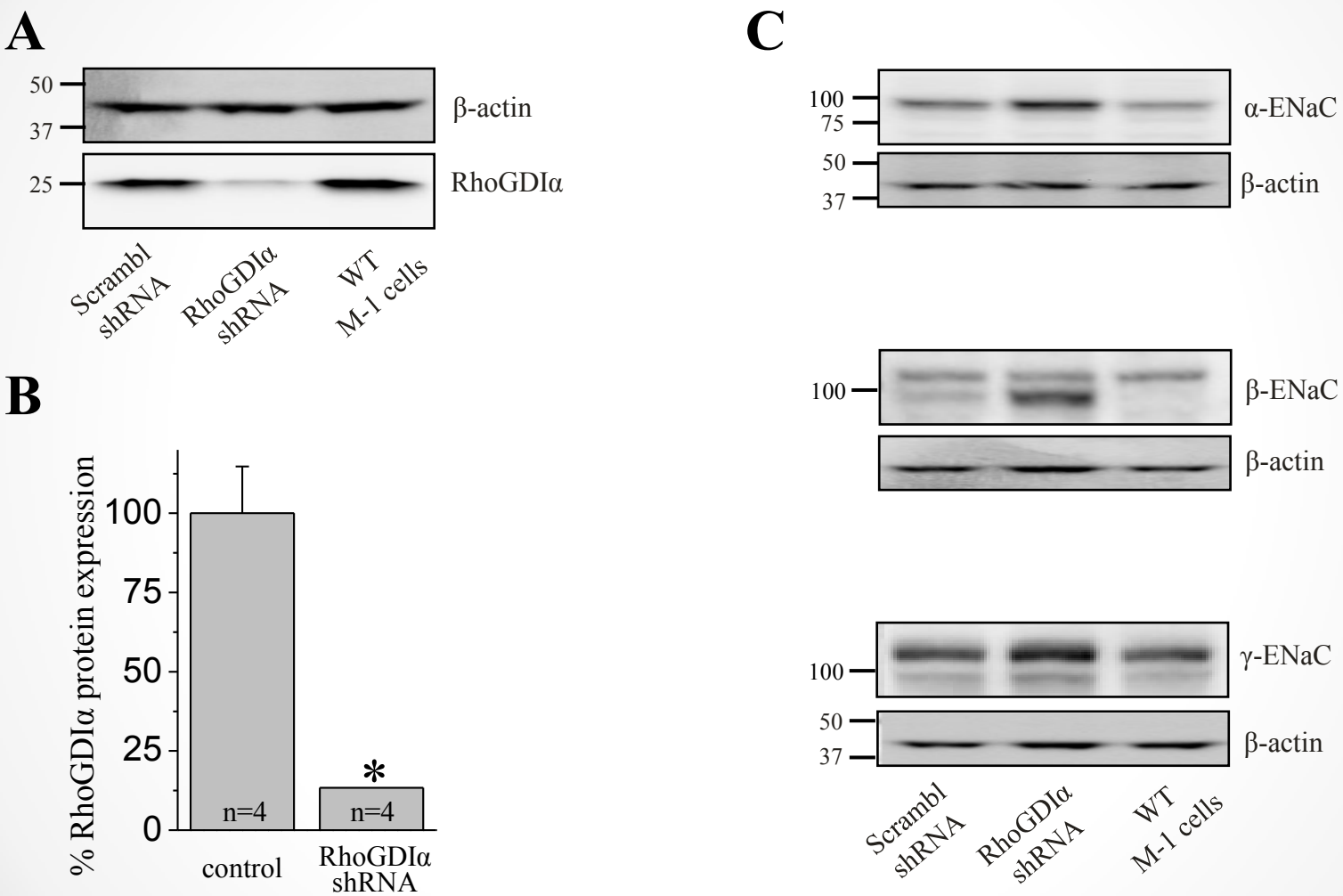


RhoGDIα downregulates ENaC activity via Rac1





Knockdown of RhoGDIα in M-1 cells upregulates ENaC expression



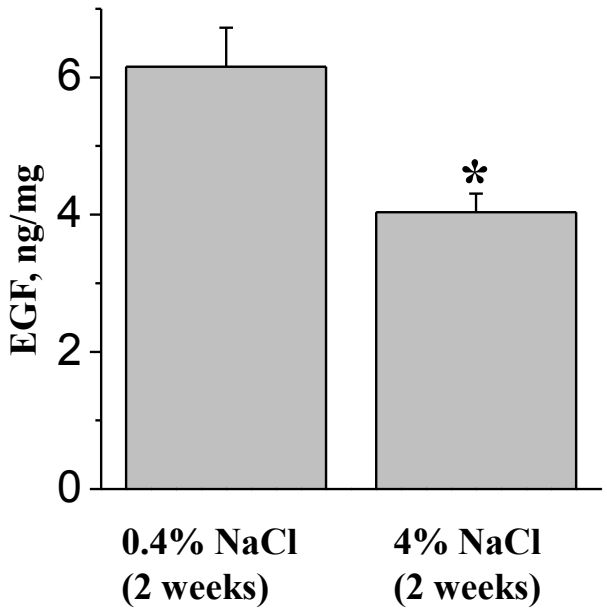
Conclusions:

Chronic treatment with EGF increases RhoGDIα expression

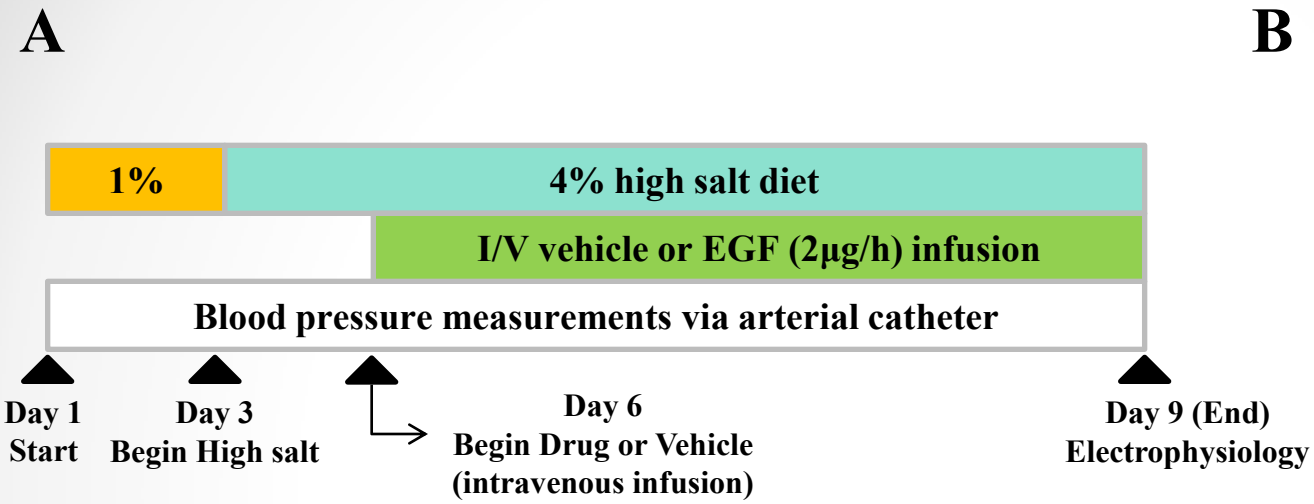
RhoGDIα downregulates ENaC activity via Rac1

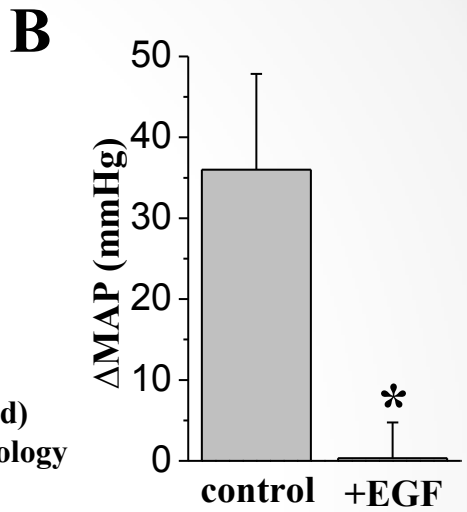
- **Role of ENaC in the development of salt-sensitive hypertension in the Dahl Salt-Sensitive (SS) rats**
- **EGF and its related growth factors regulate ENaC**
- **Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins**
- **EGF might regulates ENaC via Rho GDP-dissociation inhibitor RhoGDIα**
- **EGF modulates ENaC-mediated Na^+ transport in the ASDN and participate in the development of salt-sensitive hypertension**

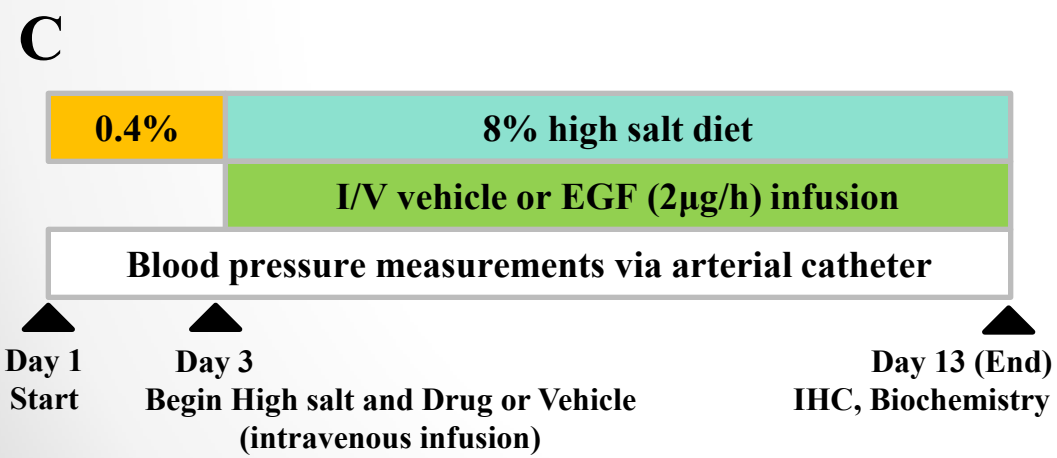
Level of EGF measured by ELISA in the cortex of SS rats fed a normal and high salt diets

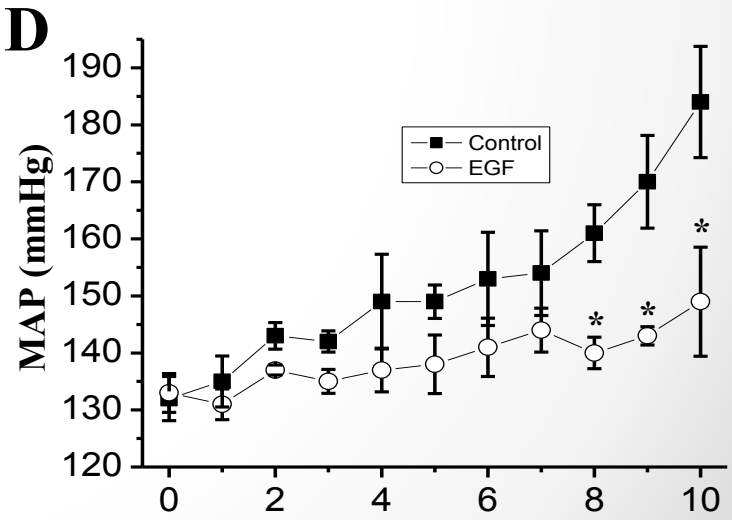


Chronic intravenous infusion of EGF prevents development of hypertension in SS rats fed a HS diet

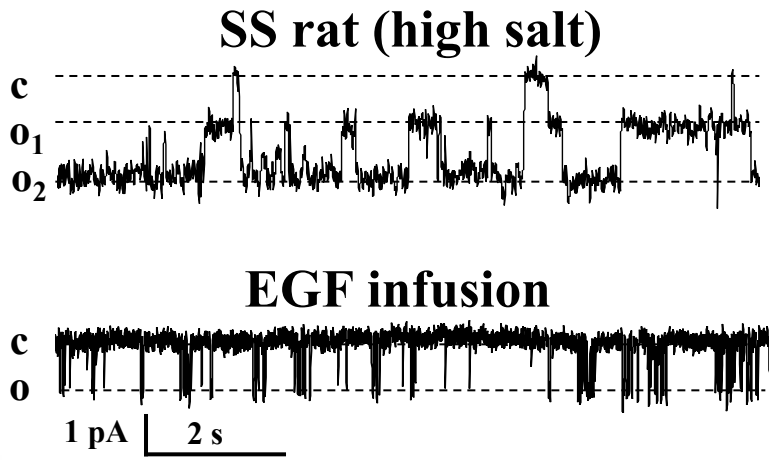


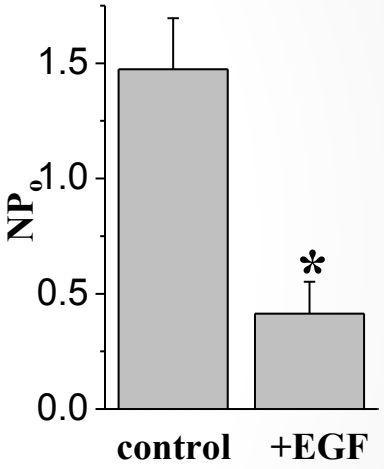


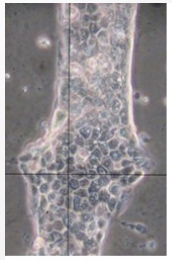


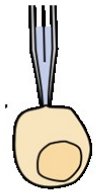


Chronic intravenous infusion of EGF decreases ENaC activity in SS rats fed a high salt diet









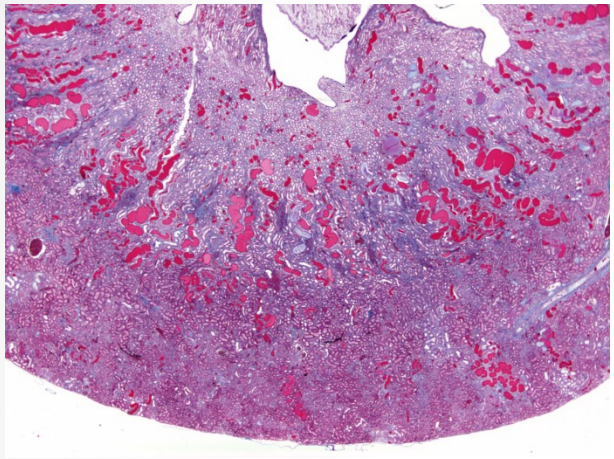
Cell-attached

EGF attenuated renal glomerular and tubular damage

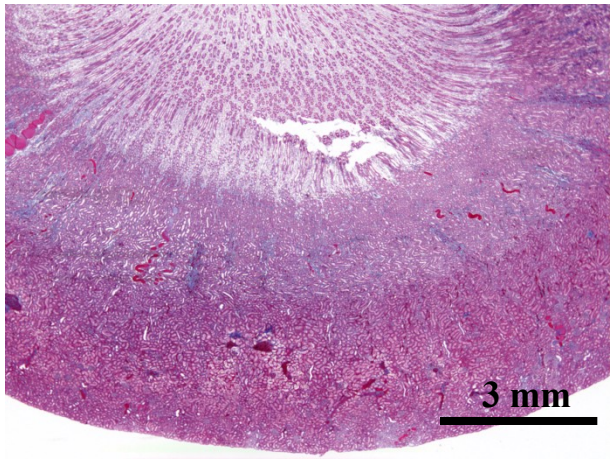
A

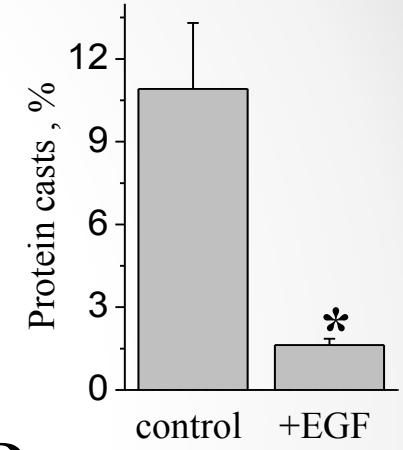
B

C

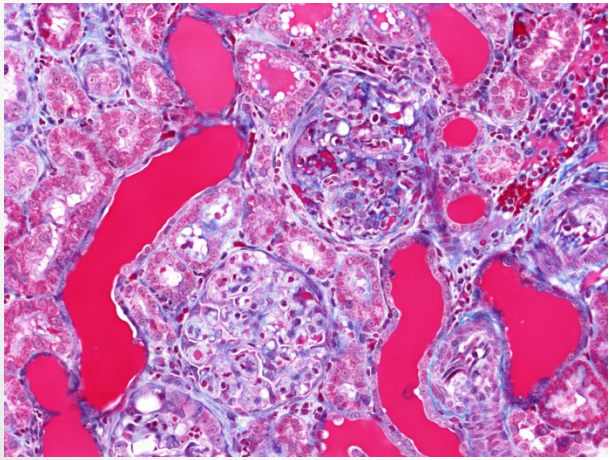


1x

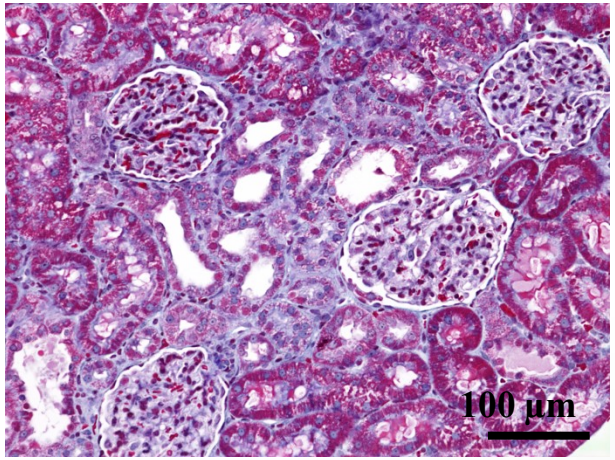


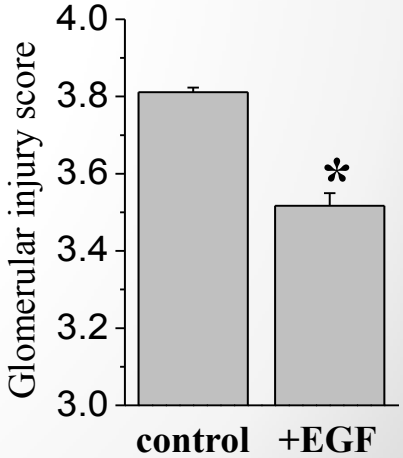


D



20x





SS rat (8%; 2 weeks)

SS rat (8%; 2 weeks; + *i/v* EGF)

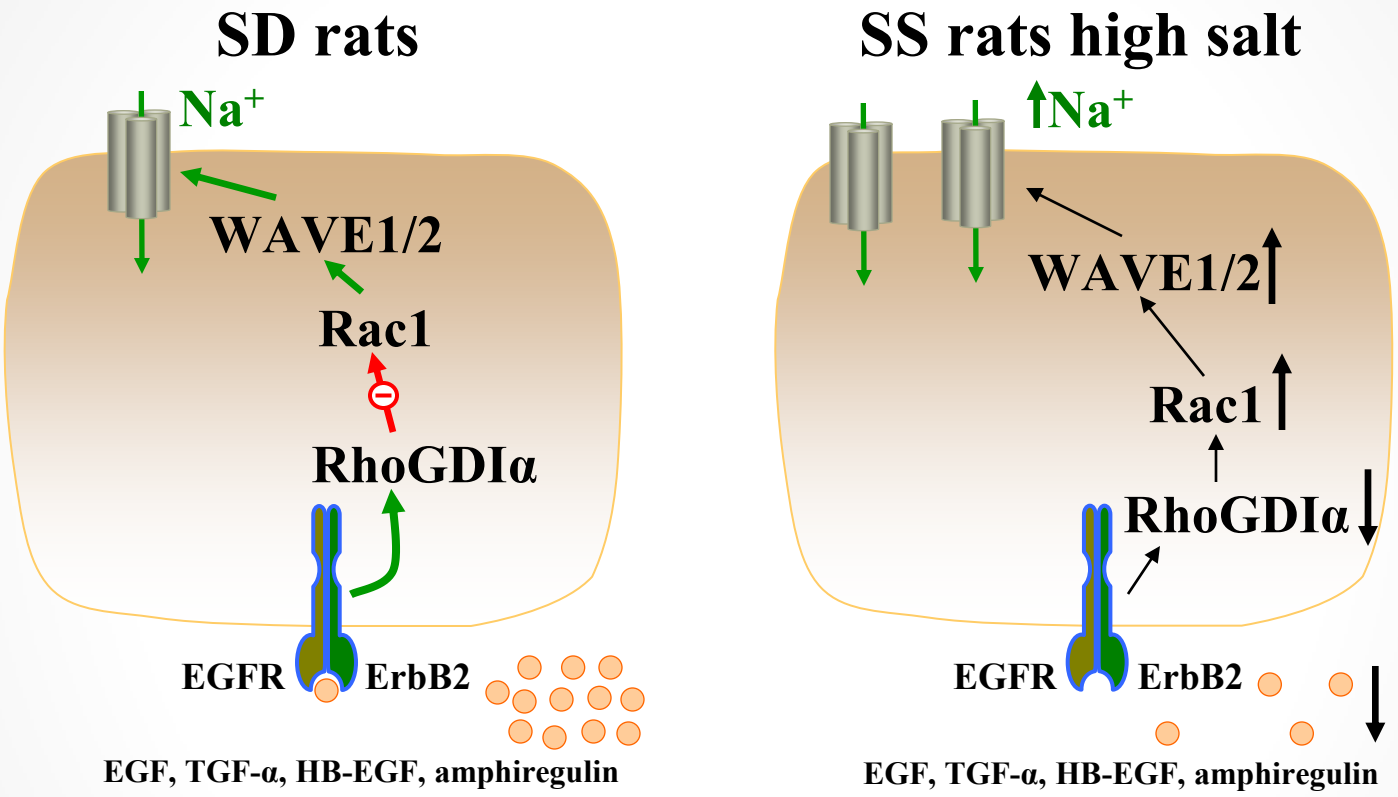
Conclusions:

EGF concentration is reduced in the SS rats fed a high diet, which we propose would enhance ENaC activity, sodium retention and hypertension.

Elevation of EGF is associated with diminished sodium reabsorption, an attenuation of hypertension, and a reduction of renal damage in Dahl SS rats fed high salt.

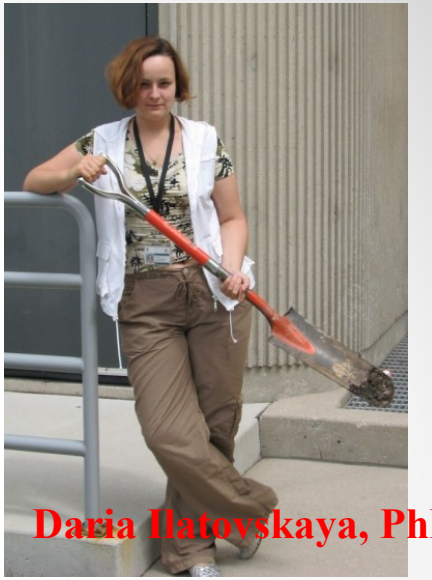
EGF deficiency in the renal cortex contributes to salt-sensitive hypertension in Dahl SS rats via upregulation of ENaC activity

Thus, elucidation of the interaction between EGF, small GTPases and ENaC will advance our understanding of the basic mechanisms of sodium regulation in salt-dependent forms of hypertension and might provide novel targets and strategies for their treatment.



Na^+ retention → Blood volume → Cardiac output → Arterial pressure

Thanks to:



Allen W. Cowley, Jr., PhD
Julian Lombard, PhD
Andrey Sorokin, PhD
David L. Mattson, PhD
Nadezhda Zheleznova, PhD
(MCW)

Paul M. O'Connor, PhD (GHSCU)
Patricia D. Wilson, PhD (UCL)

Research was supported by the American Heart Association,
American Diabetes Association and NIH Heart, Lung and Blood Institute
Отдельное спасибо фонду Династия