ATP dependency of osmotically activated outwardly rectified current in the membrane of cytoplasmic droplets obtained from sporangiophore of model filamentous fungus *Phycomyces blakesleeanus*

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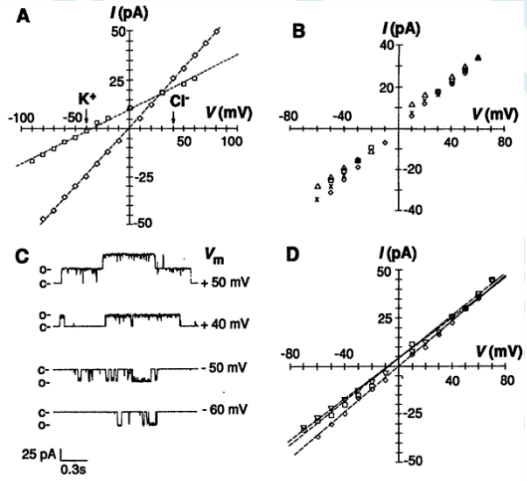
Министарство просвете,
науке и технолошког развоја

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Only 6 cell membrane ion currents are characterized electrophysiologically in all filamentous fungi

Cell wall digestion

Mechanosensitive channel in germlings of *Uromyces appendiculatus*



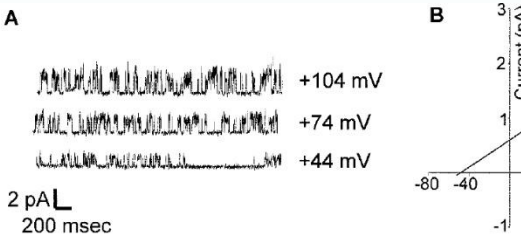
Zhou at al., Science (1991), Science 253: 1415-17

Laser ablation of cell wall

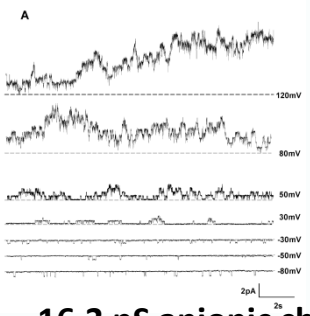
43 pS plasma membrane anion efflux channel from *Aspegillus niger* hyphal membrane
(Roberts et al. *(1997), New Phytol. 137, 579-585)*

Heterologous expression in yest

16pS K^+ outward rectifier from *Neurospora crassa* hyphal membrane
(Roberts et al. (2003) Eukaryot Cell. 2: 181-190)

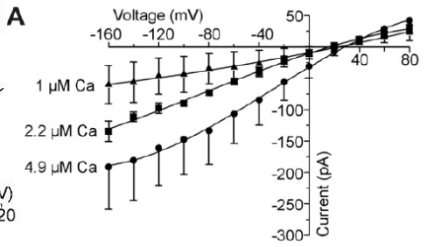


Cytoplasmatic doplets *P. blakesleeanus*

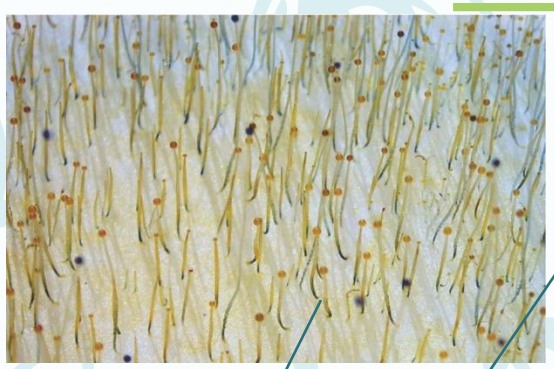


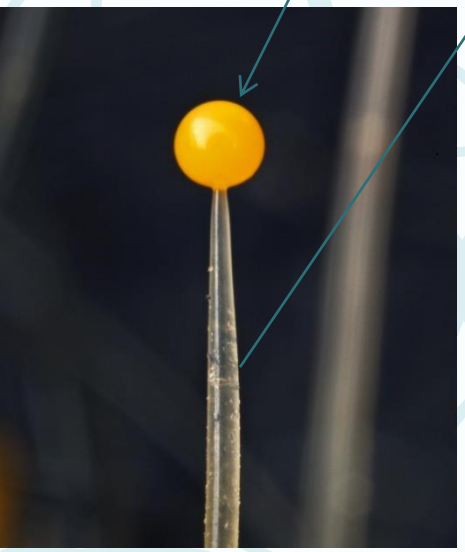
Two anionic channels
(ORAC, Živić et al. (2009), Eukaryot Cell. 8: 1439-48 and ORIC, Križak et al., (2015), Res Microbiol. 166:162-73)

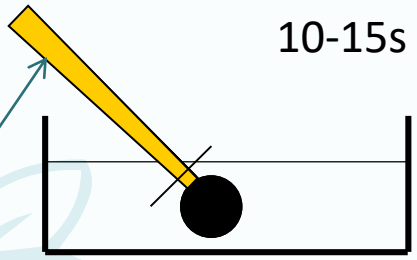
16.3 pS anionic channel from *A. nidulans* hyphal membrane
(Roberts et al. (2011) Fungal Genetics and Biology 48: 928–938)



Our model system is membrane obtained from aerial hyphal cell – sporangiophore of *Phycomyces blakeseeanus*

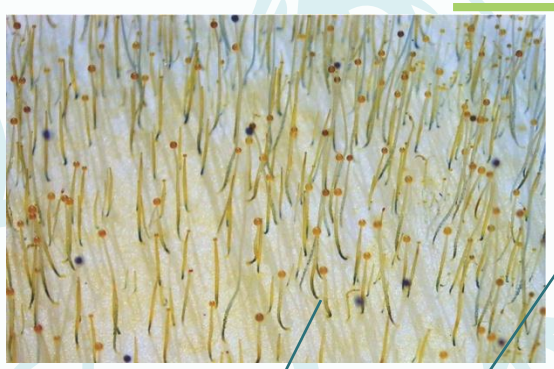


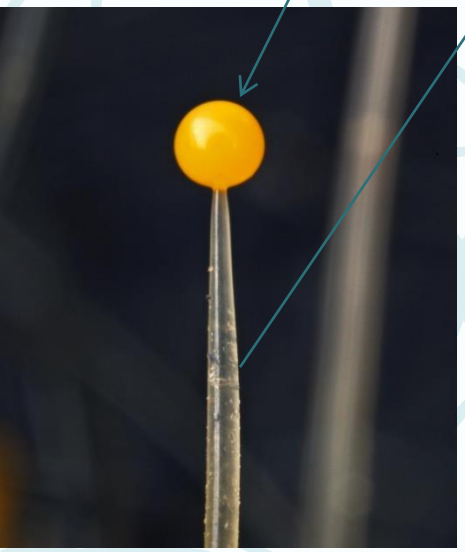


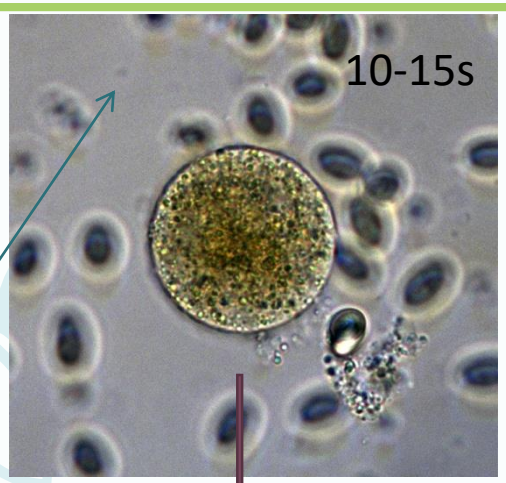


few hours

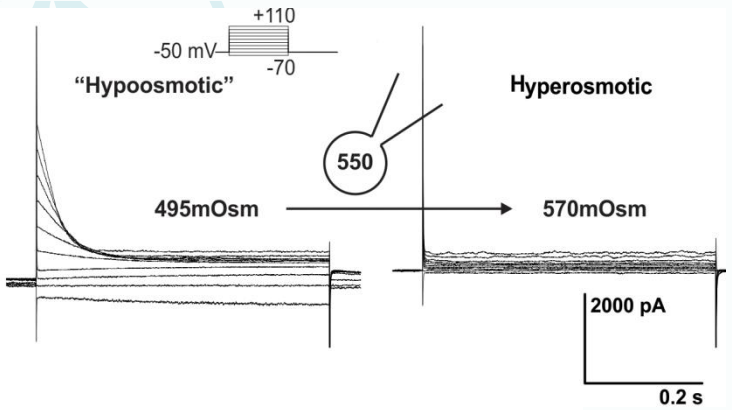
Our model system is membrane obtained from aerial hyphal cell – sporangiophore of *Phycomyces blakeseeanus*

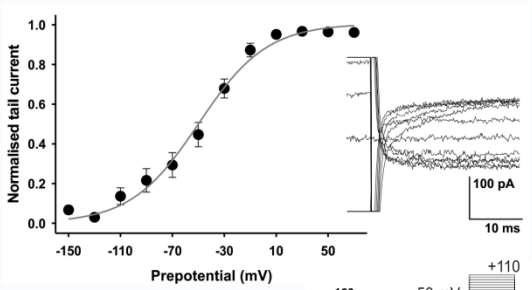


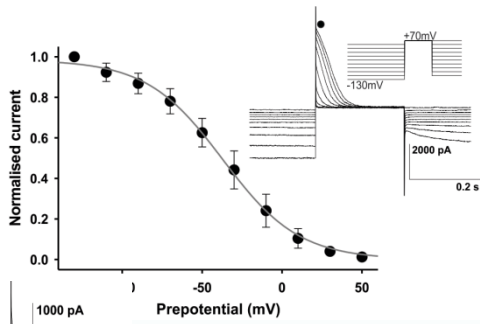


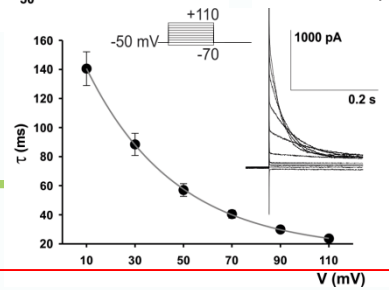


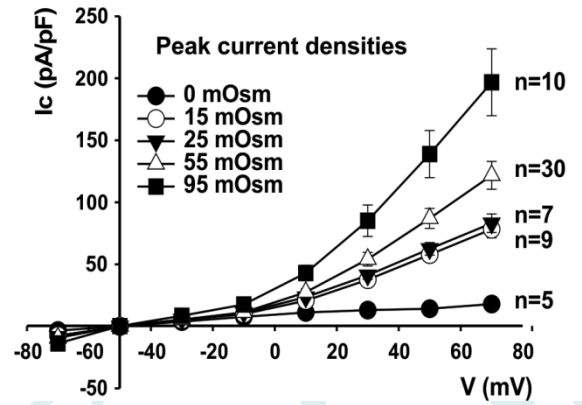
few hours









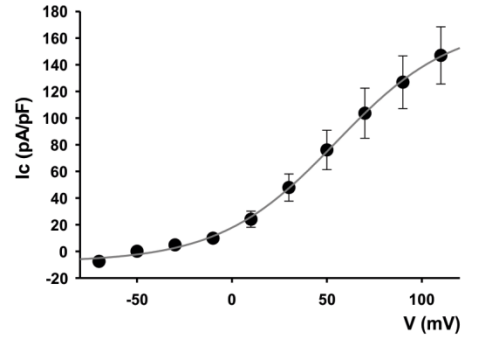


ORIC ≈ VRAC

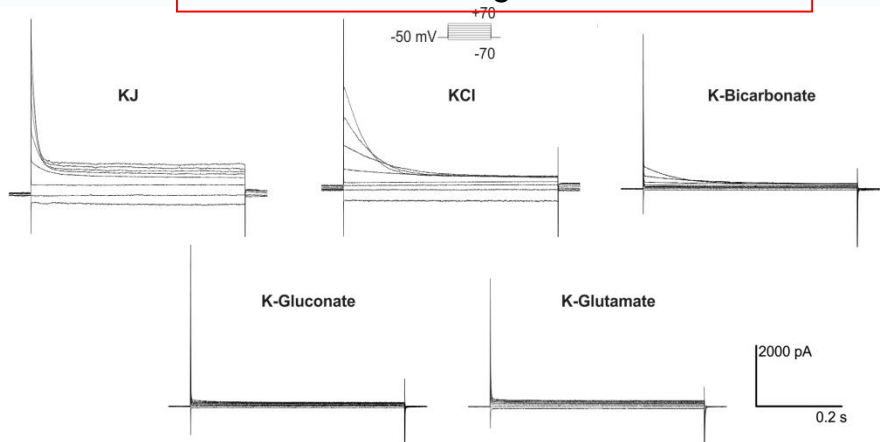
voltage and time-dependent inactivation at positive potentials and recovery from inactivation at negative potentials

activation by osmotically induced swelling

permeability sequence:
$I^- > Cl^- > HCO_3^- >$ gluconate

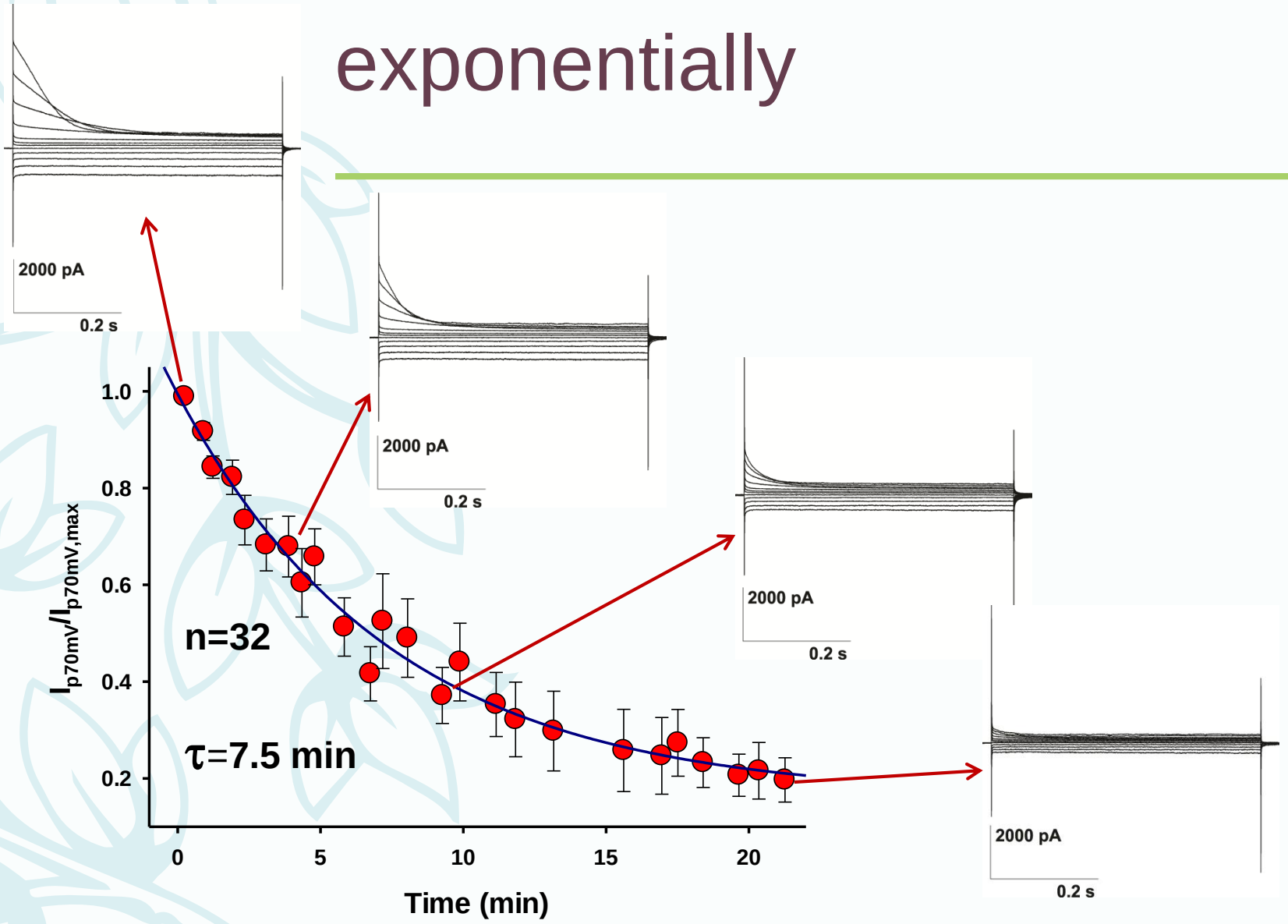


moderate outward rectification

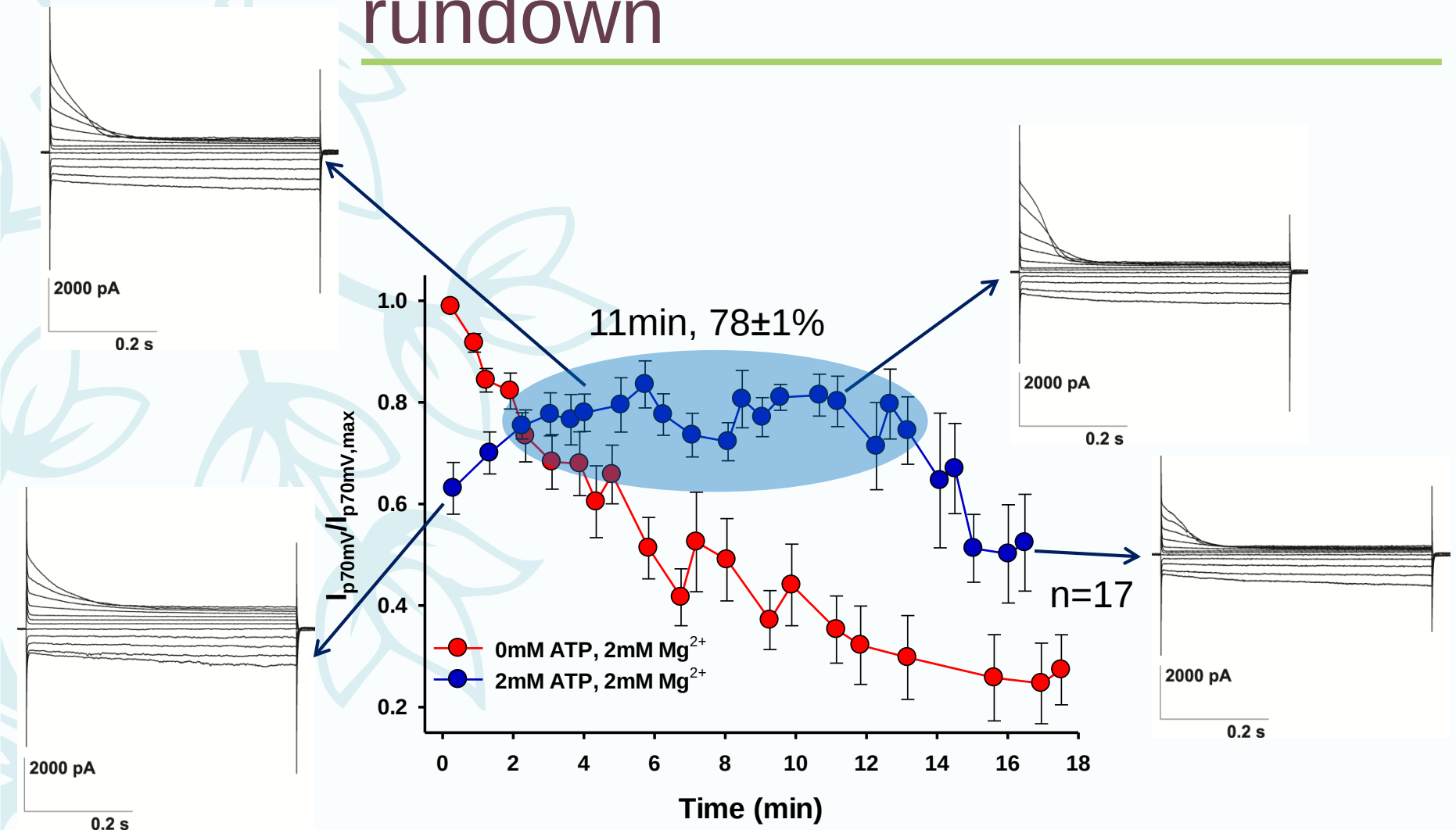


Križak S, Nikolić L, Stanić M, Žižić M, Zakrzewska J, Živić M, Todorović N., Res Microbiol. 2015 166:162-73.

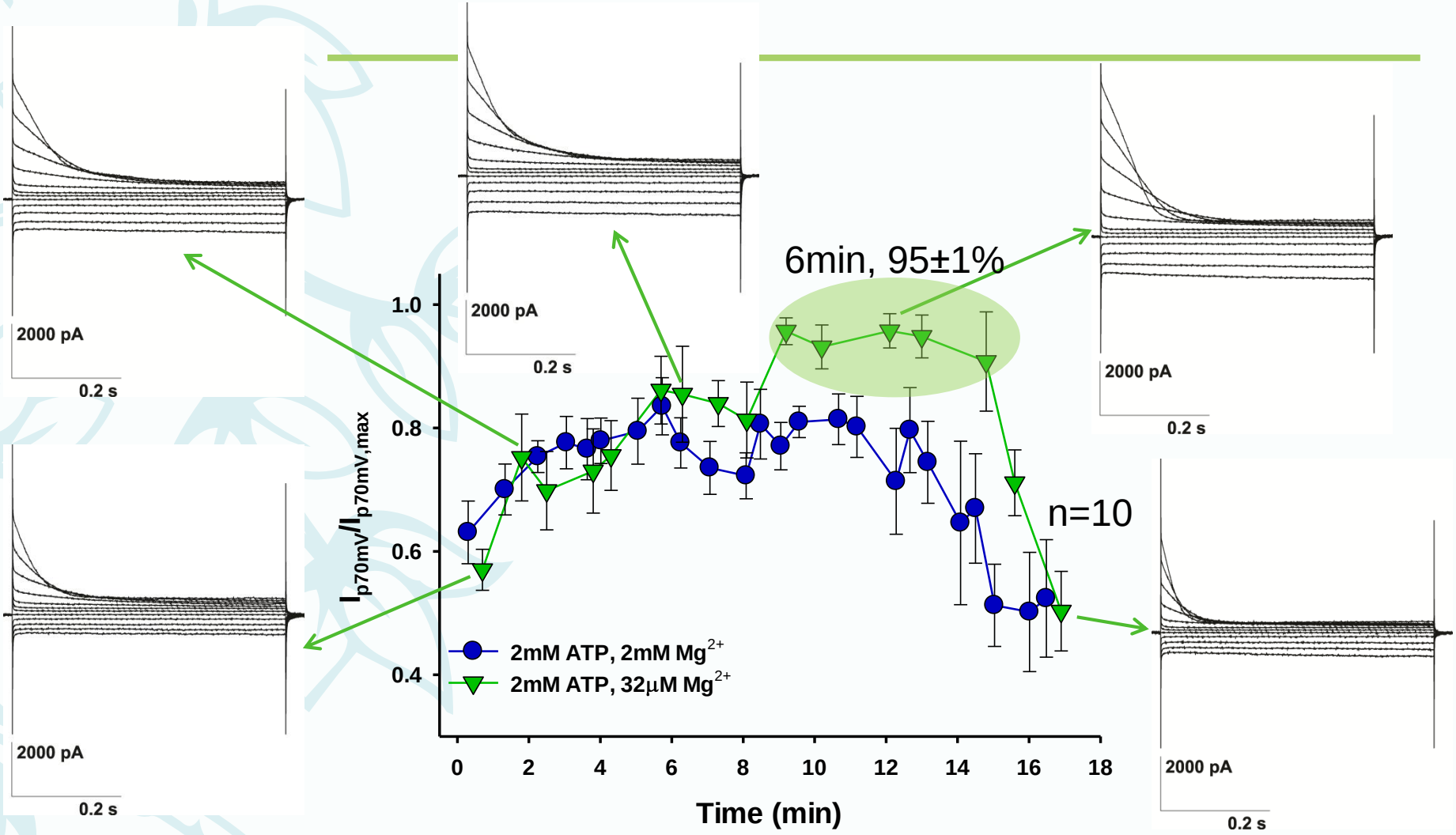
Without ATP ORIC rundown exponentially



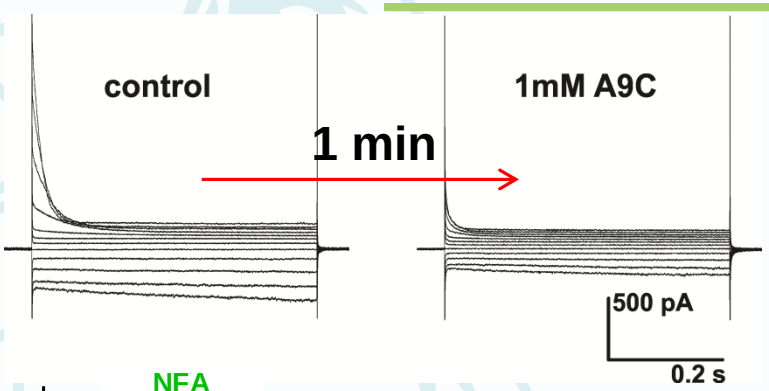
2mM intracellular ATP significantly slows ORIC rundown

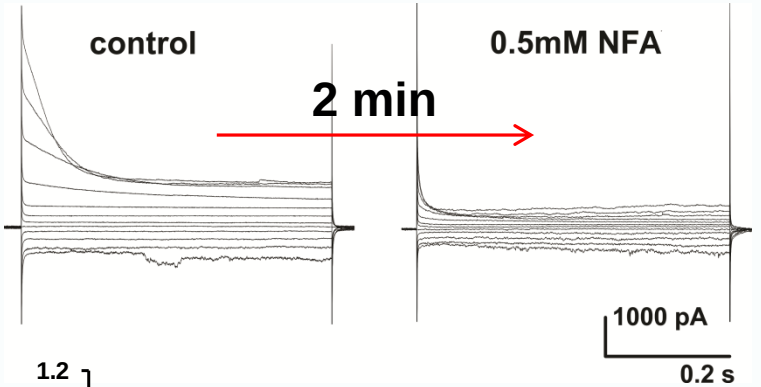


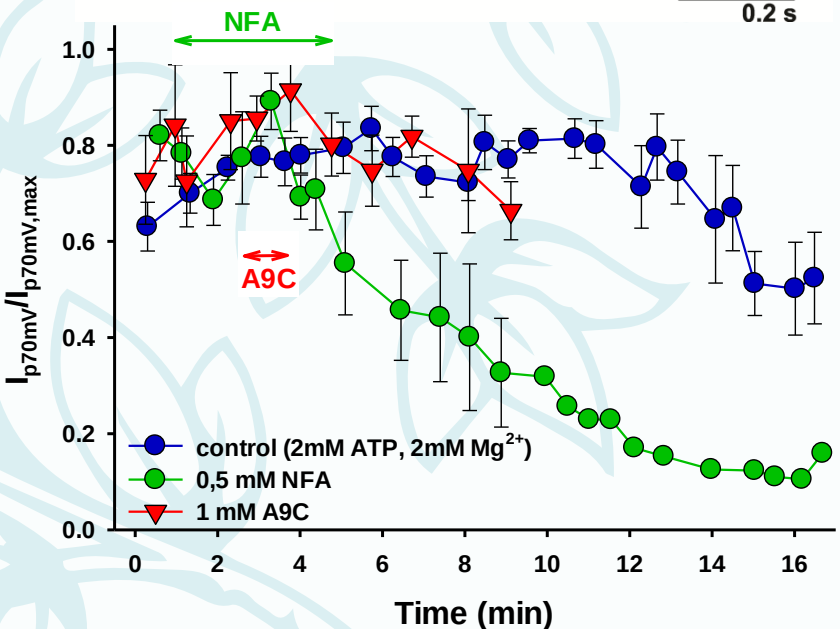
Mg^{2+} reduction further activates ORIC

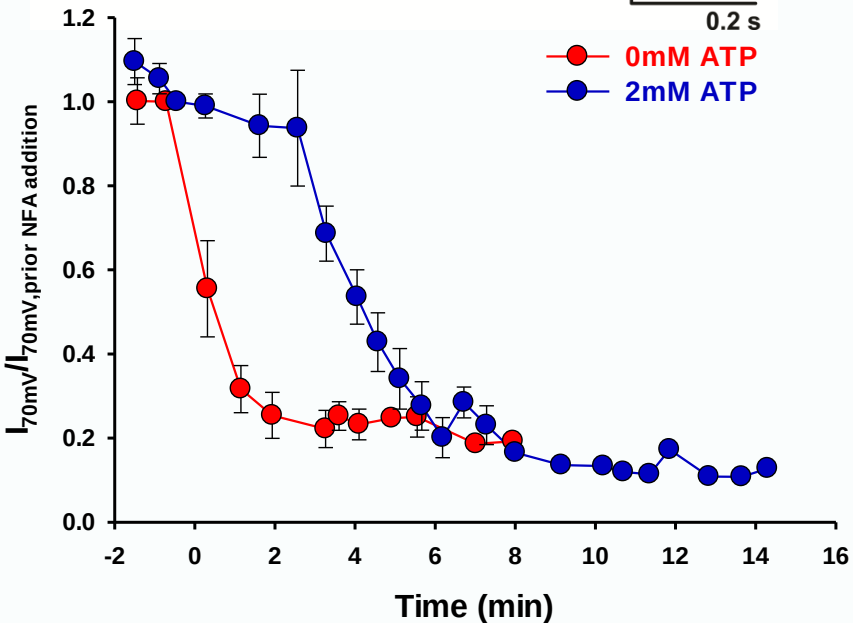


ATP modifies effects of ORIC inhibitors, A9C and NFA

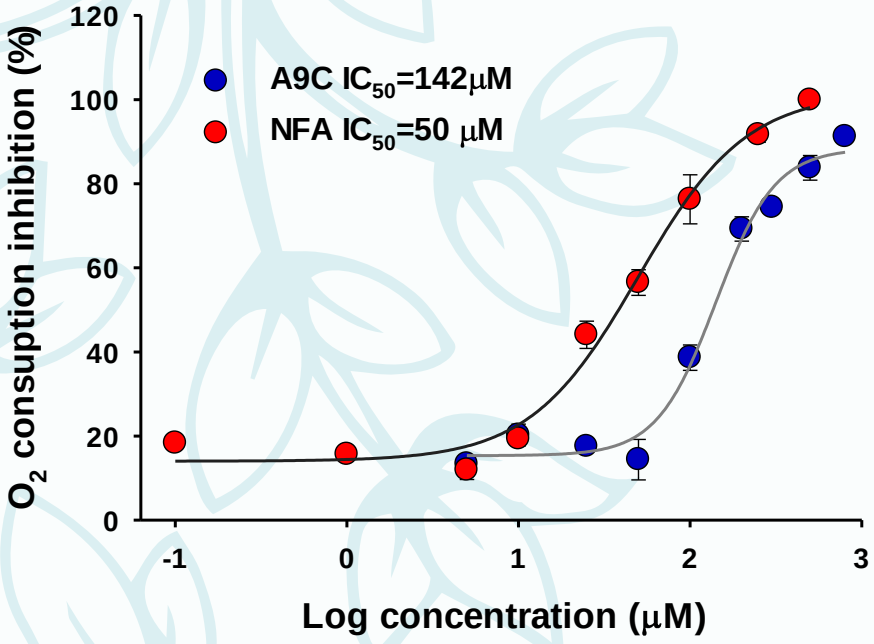


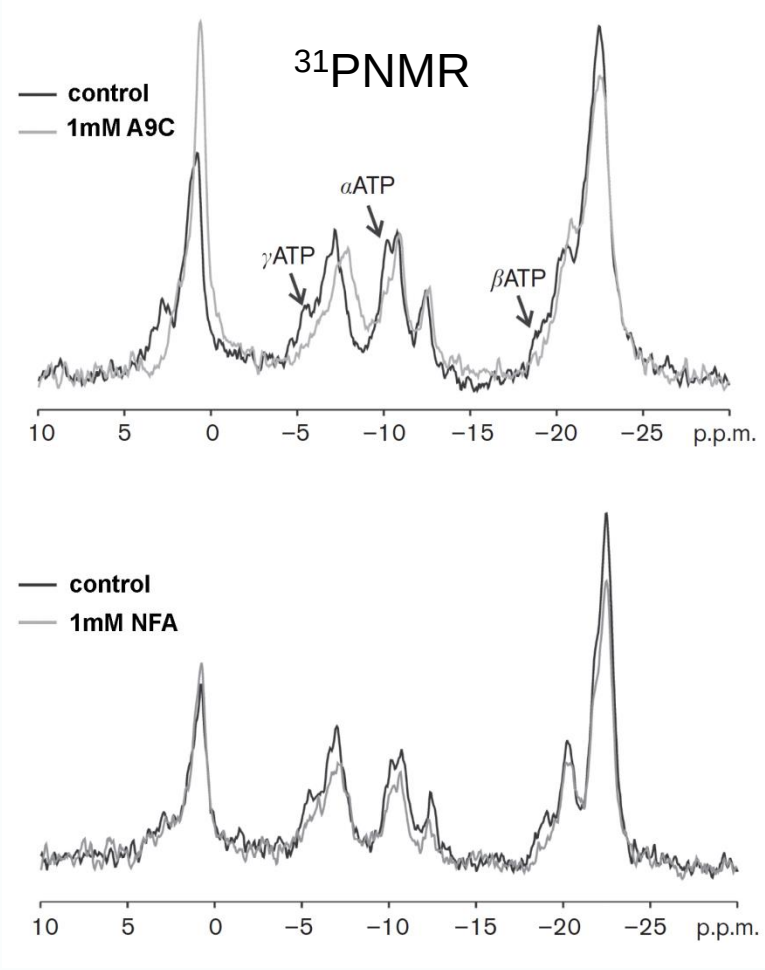




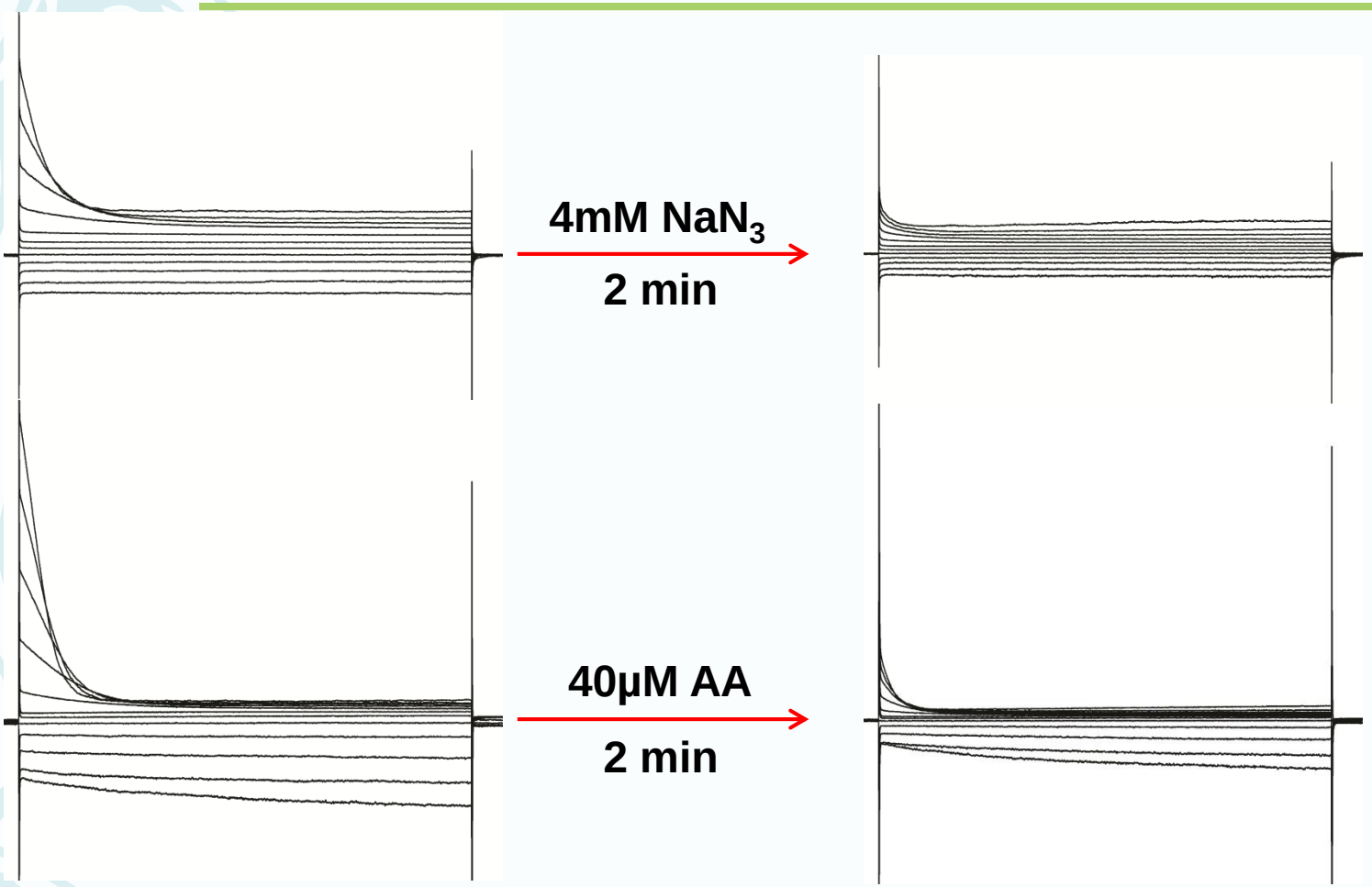


A9C and NFA are potent inhibitors of cell respiration and ATP synthesis





Cell respiration inhibitors, azide and Antimycin A block ORIC



Conclusions

- Intracellular ATP is necessary for ORIC activity
- Intracellular Mg^{+2} exert an inhibiting effect on ORIC
- Primary effect of NFA and A9C on ORIC is probably indirect via reduction of intracellular ATP concentration.