SC^2P

Standard Calcium & Channel Screening

(this protocol must be used with the C^2EP protocol A&B)

Notes: To ensure a fast, reproducible and fast screening of transfected/infected cells on Ca^{2+}/channel signaling the following standard protocols should routinously repeated at least on three different days or with at least three different cell patches.

Data Acquisition:

1. Make sure that you recieved all information necessary to complet the CCEP. No exceptions!
2. Carefully explain the experimental parameters. The time you win not do so is minimal but you save hours or days afterwards.

Data Analysis:

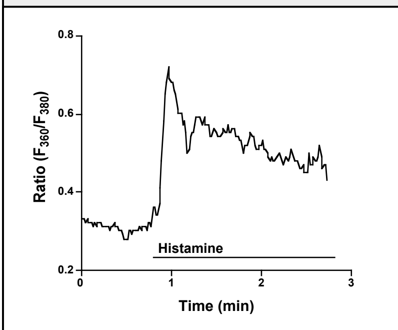
1. Perform data analysis immediatly!
2. Analyse first, be critical to our hypothesis and try to extract as many as possible information out of the experiments (e.g. basal Ca^{2+}, amplitude of release, area under the curve, time correlation, etc.). Never forget that subpopulations are possible particular in trasnfected cells (e.g. different amount of protein expression).

Standard Screening Protocols

Standard Protocol 1: "Normal Ca^{2+} protocol"

Puffers used: ✓ **2NaCa** ❍ ~~0CaNa~~ ❍ ~~0CaNa, EGTA~~

After 1 min experimental time to monitor basal Ca^{2+} concentration add the agonist to be tested (e.g. 10 μM histamine). Wait until a constant plateau or a clear and constant decline to basal level is shown. Do not stop too early - You might loose information!



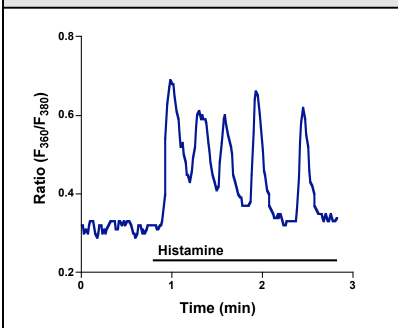
Data analysis:

- ■ Check basal Ca^{2+} levels (is it constant or changing?)
- ■ Calculate raise in Ca^{2+} in response to the agonist. Don't forget to compare even the onset time and speed of elevation between given groups.
- ■ Calculate Ca^{2+} plateau (if there is one) or the time constant for decline (τ= half time until the Ca^{2+} concentration reaches the resting level)
- ■ Any oscillations or other events during onset, plateau or decline?

Standard Protocol 2: "Ca^{2+} Oscillation protocol"

Puffers used: ✓ **2NaCa** ✓ **0CaNa** ❍ ~~0CaNa, EGTA~~

After 1 min in Hepes+2.5 mM Ca_e switch to Ca^{2+}-free solution and monitor basal Ca^{2+} over additional 1 min. Thereafter add the agonist (e.g. 10 μM histamine) for 2 to 3 min. Wait until oscillation stops or Ca^{2+} returns to basal. Do not stop too early - You might loose information!



Data analysis:

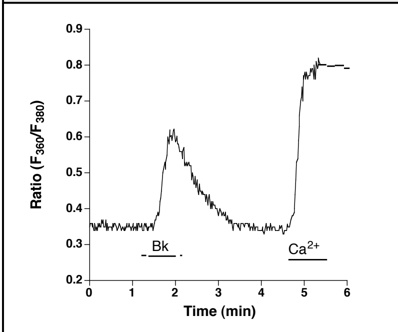
- ■ Check basal Ca^{2+} levels (is it constant or changing?)
- ■ Calculate 1st raise in Ca^{2+} in response to the agonist. Don't forget to compare even the onset time and speed of elevation between given groups.
- ■ Calculate Ca^{2+} threshold for oscillation (if there is one), the amplitude and the frequency.
- ■ Check the kinetics of oscillation by duration of Ca^{2+} elevation, τ, etc.

Department of Medical Biochemistry, University of Graz, Austria
wolfgang.graier@kfunigraz.ac.at

Standard Protocol 3: **"Ca^{2+} Release protocol"**

Puffers used: ✓ **2NaCa** ❍ ~~0CaNa~~ ✓ **0CaNa, EGTA**

After 1 min in Hepes+2.5 mM Ca_e switch to Ca^{2+}-free solution and monitor basal Ca^{2+} over additional 1 min. Thereafter add the agonist (e.g. 10 μM histamine) for 1 to 2 min. Wait until Ca^{2+} returns to basal level (app. 3 to 4min) and add Hepes + 2.5 mM Ca_e (app. time point 6 min) Do not stop too early! Wait until after addition of Ca_e a plateau has been reached or Ca returns to baseline!



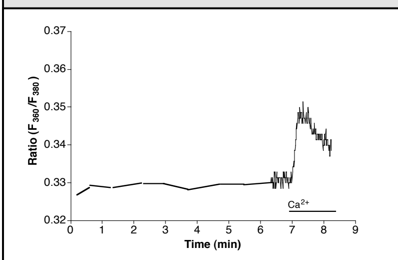
Data analysis:

■ Check basal Ca^{2+} levels (is it constant or changing?)
■ Calculate Ca^{2+} transient in response to the agonist. Don't forget to compare even the onset time, area under the curve and speed of elevation/decline between given groups.
■ Calculate the raise in Ca^{2+} in response to the addition of Ca_e. Check the kinetics of this increase and whether plateau or decline.

Standard Protocol 4: **"Ca^{2+} Leak protocol"**

Puffers used: ✓ **2NaCa** ~~0CaNa~~ ✓ **0CaNa, EGTA**

After 1 min in Hepes+2.5 mM Ca_e switch to Ca^{2+}-free solution and monitor basal Ca^{2+} over additional 4 to 5 min. Add Hepes + 2.5 mM Ca_e (app. time point 5 to7 min). Do not stop too early! You might loose information!



Data analysis:

■ Check basal Ca^{2+} levels (is it constant or changing?)

■ Calculate raise in Ca^{2+} in response to the addition of Ca_e. Check the kinetics of this increase and whether plateau or decline.

Data process and handling:

1. Store your original printouts and the protocols together with the analysis of the data.

2. Provide WFG a Prizm printout and the file with your analysis via e-mail.

3. Data analysis must be provided with given mean ± sem (and n!).

4. Provide statistical evaluation using the Prizm program (unpaired t-test). Level of significance should be hold at $p<0.05$.

5. Stay in contact with other team members on their effort. Talk and discuss your findings with all the perople in the team and tell them your findings. Do not hide but show your data. Just discussion makes us using forward.

Department of Medical Biochemistry, University of Graz, Austria
wolfgang.graier@kfunigraz.ac.at