Blog of a 2022 Summer internship at Dr Moss and Benton Lab

One of the K+ channel subgroups are channels activated by Ca^{2+}-activated K^+ channels often subdivided into IK, SK, and BK channels. In my BPS-sponsored placement, I was transfecting HEK 293 cells with a human BK channel and the auxiliary subunit β4 (present in neurons). and I was recording currents assumingly flowing only through that channel. I was testing the effect of drugs BK channel activators VSN16R, ketoconazole and derivatives. Inside-out and whole-cell patches were used to record currents.

VSN16R

VSN16R is a drug in a phase 2 clinical trial for treating spasticity in multiple sclerosis. Although studies in animal models were very promising the subunit selectivity of VSN16R in activating BK channels were not tested before. Interestingly, VSN16R increased channel steady-state currents by having a massive impact on K^+ channel activation kinetics. Activation by VSN16R can be seen below (Fig. 1).

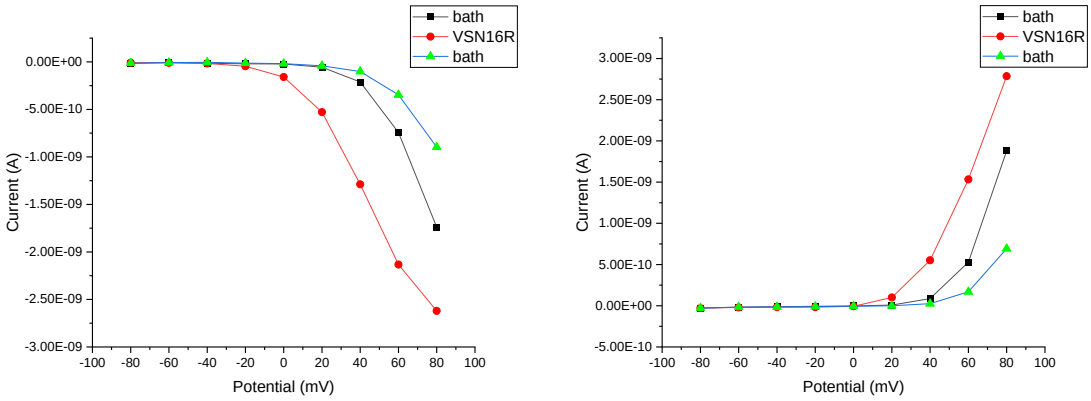

Figure 1. Currents recorded using the inside-out patch clamp technique in the bath with nominally zero Ca^{2+} are plotted against the membrane potential at which the membrane was held. A) tail current graph, B) steady state current graph (9/8/2022).

Tail current is a current flowing through a BK channel after the voltage set by the amplifier is such that does not active BK channels anymore. The channel closes due to this voltage being below the activation threshold, but it does so slowly (compared to the electronics) because it takes time for channel proteins to change their conformation. The bigger the tail current, the more current is flowing via a channel and over time the tail current gets smaller.

Ketoconazole

I used ketoconazole (called keto in the graph) in addition experiments, again with the idea of identifying subunit selectivity. I hypothesized that ketoconazole will not work on BK channels with β4 subunits. Results showed that ketoconazole increased steady-state activation are shown below (Fig. 2).

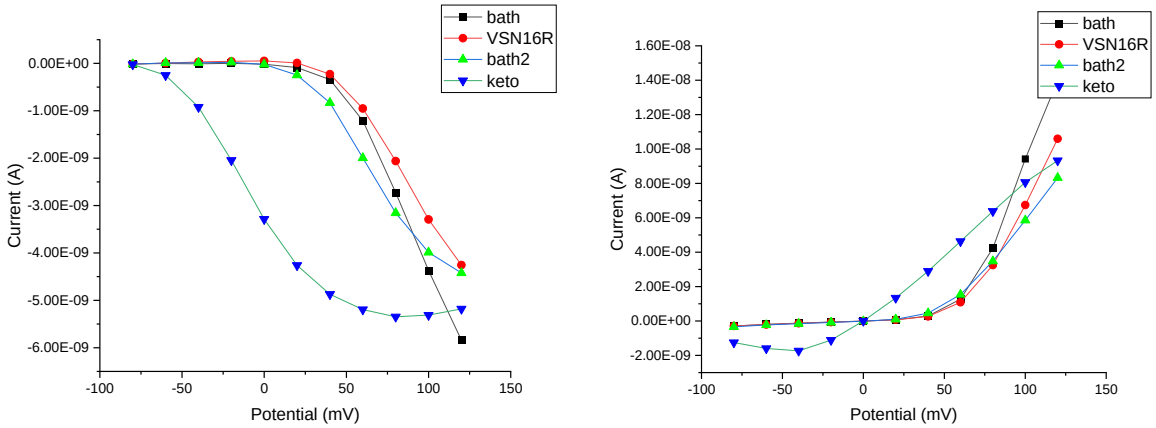

Figure 2. Currents recorded using the inside-out patch clamp technique in the bath with 0 Ca^{2+} are plotted against the membrane potential at which the membrane was held. A) tail current graph, B) steady state current graph (9/8/2022).

Ketoconazole also altered BK channel kinetics. Figure 3 indicates that ketoconazole allowed faster channel opening (altered k_{+1}) and slowed channel closure with the channel thus spending more time in the open state.

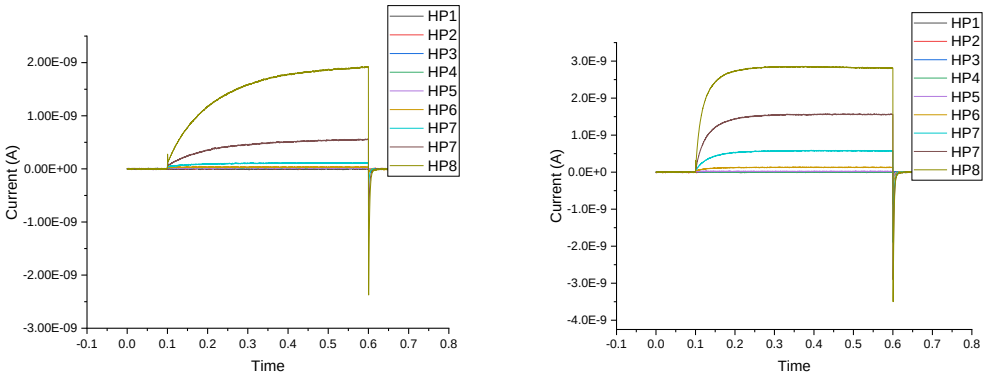

Figure 3. Currents were recorded at various holding potentials (HP) A) in the control and B) in ketoconazole group experiments (8/9/2022).

Control experiments

Described experiments with VSN16R β4 subunits were performed on cells without β4. As expected, VSN16R did not work on most cells lacking β4. Because the β4 subunit is neuronal, after applying VSN16R on neurons, the BK channel should faster open but without much change in the closing rate.

Results showed that in nominally 0 free Ca^{2+} solutions the current-voltage relationship shifts to the right compared to 1 µM free Ca^{2+} solution. This indicates that in 0 free Ca^{2+}, a much higher potential is required to open the channels than in 1 µM free Ca^{2+} solution (Fig. 4).

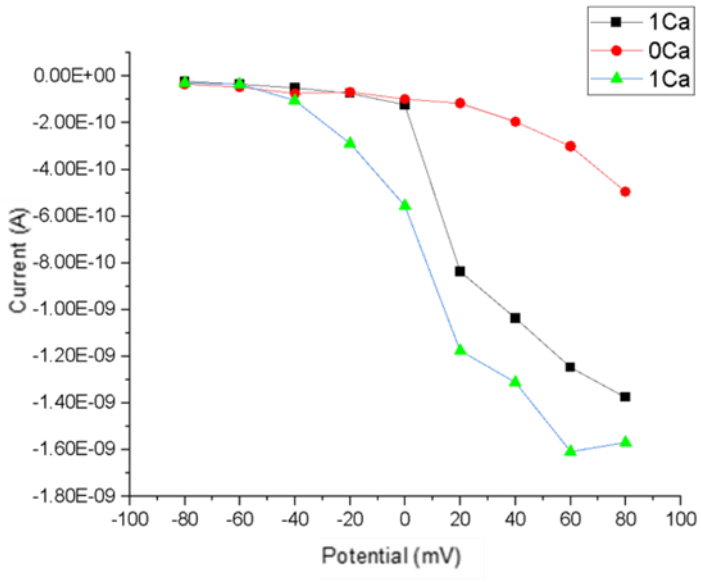

Figure 4. Steady-state currents recorded using the inside-out patch clamp technique in the bath with 1 or 0 free Ca^{2+} are plotted against the membrane potential at which the membrane was held (18/7/2022).

Experience

When inside-out patches stopped working reliably, I experienced that patch clamping is a technically hard technique. I learnt that support from senior people is very important in the lab, and I was very lucky to have bright and supportive supervisors. I was also happy to get to know other PhD students and an intern in this lab. I am certain that these connections and data processing skills will be beneficial in the future. I especially liked plotting graphs in the software ORIGIN which is similar to Excel but graphs plotted in ORIGIN look much more evident. Overall, I would highly recommend that students do a research internship during the summer because it is a great opportunity to both network and learn something beyond what is taught in class during the academic year.

Acknowledgement

I thank the British Pharmacological Society for funding me to perform this project and I am grateful to Dr Moss and especially Dr Benton for their time and valuable lessons.