Persistent Neuropathic Pain:

Do Voltage-Gated Potassium Channels and Calcium Sensing Receptors Crosstalk?

Determining functional and molecular interaction between Kv7 and CaSR and their roles in persistent neuropathic pain

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Introduction

Persistent neuropathic pain is a condition caused by hyperexcitability of neurons responsible for pain signalling due to nerve injury or inflammation[1]. Most approach to treatment focus on targeting sodium and calcium ions from entering the cell. However, potassium also plays a crucial role in repolarising the cell.

Dysfunction of voltage-gated potassium channels (Kv7) prevents potassium exiting the cell which leads to increased firing of pain signals in the neurons. Inflammation can stimulate Calcium sensing receptors (CaSR), preventing restoration of normal calcium levels inside the neuron[2]. This, together with nerve injury, can cause excessive calcium ion accumulation, with development of persistent pain and consequent cell death[3].

Our hypothesis is that CaSR and Kv7 are functionally linked together. Therefore, in this study we investigate if stimulating or blocking of CaSR have any effects on Kv7 channels current and consequently the cell's excitability.

Aims

- Determine whether high calcium and CaSR pharmacology have any effects on Kv7 channel current and membrane potential
- Explore the crosstalk between Kv7 and CaSR through Gq-PLC pathway

Methods

Cells cultured

- Chinese Hamster Ovarian cells stably expressing Kv7 channels (CHOKv7.2/7.3)
- Human Embryonic Kidney cells stably expressing CaSR (HEK293 CaSR)

Immunocytochemistry

CHOKv7.2/7.3 and HEK293 CaSR were stained with green (CaSR), red (α-Tubulin) and blue (nucleus) to confirm expression of CaSR.

Patch-clamp

- Kv7 current and membrane potential of the cells were recorded real-time
- Different pharmacology was added to the bath with the cells

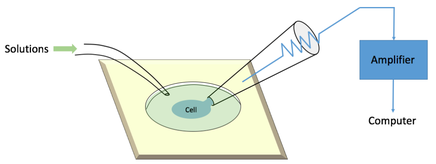

Figure 1. Scheme of experimental chamber

CaSR	Kv7	PLC
• High Calcium ([Ca^{2+}] 5.0mM) • Calcimimetic (R568) • Calcilytic (NPS2143)	• Kv7 opener (Retigabine) • Kv7 blocker (XE991)	• PLC activator (3M3FBS) • PLC inhibitor (U73343)

Results

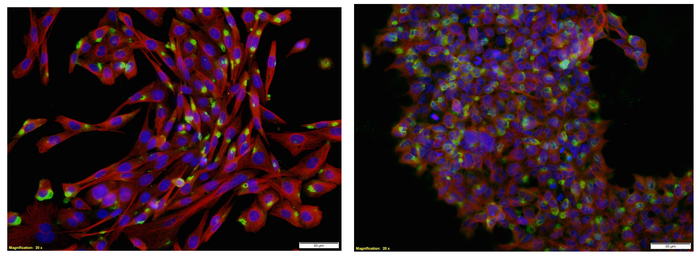

Figure 2. Expression of CaSR in CHOKv7.2/7.3 and HEK293 CaSR. Immunocytochemistry showing expression of CaSR (green) and α-Tubulin (red). Expression of CaSR by CHOKv7./7.3 is confirmed.

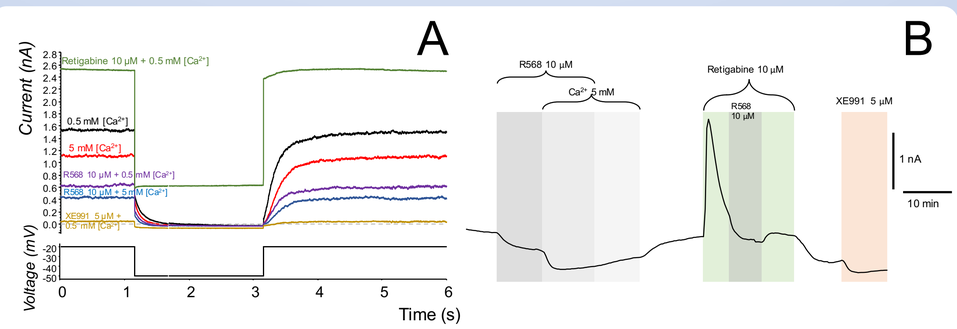

Figure 3. Effects of CaSR activation on Kv7 current in CHOKv7.2/7.3. Kv7 current in different pharmacology (upper panel); deactivation protocol with holding voltage -20mV and a step to -50mV (lower panel) (**A**). Trace of the time curse of Kv7 current recorded using deactivation protocol (**B**).

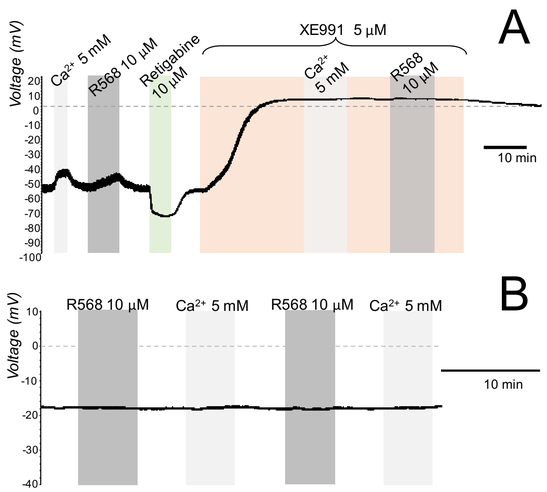

Figure 4. Comparison of effects of CaSR activation on membrane potential of CHOKv7.2/7.3 (**A**) and HEK293 CaSR (**B**)

- [Ca^{2+}] 5.0mM and R568 reversibly suppress Kv7 current and reversibly depolarise CHOKv7.2/7.3 cells
- [Ca^{2+}] 5.0mM and R568 have no effects on membrane potential of CHOKv7.2/7.3 cells in presence of Kv7 blocker (XE991)
- [Ca^{2+}] 5.0mM and R568 have no effects on membrane potential of HEK293 cells which do not express Kv7
- PLC activator and inhibitor (data not shown) have no effects on changes of membrane potential of CHOKv7.2/7.3 cells produced by [Ca^{2+}] 5.0mM and R568
- CaSR blocker decreases the membrane potential of CHOKv7.2/7.3 cells but did not change the effects of [Ca^{2+}] 5.0mM and R568 (data not shown)

Discussion

- During transition from acute to persistent pain, excessive calcium accumulates outside of the cell, stimulating CaSR
- Stimulation of CaSR inhibits Kv7 via a G-protein pathway (not Gq-PLC pathway) resulting in depolarisation which increases neuronal excitability and pain symptoms

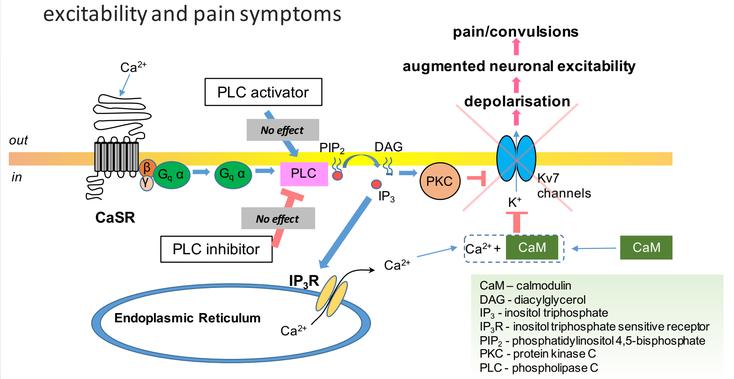

Figure 5. Diagram illustrating CaSR receptor – Gq pathway. CaSR is linked to a Gq protein which activates PLC, hydrolysing PIP_2 to DAG. DAG then activates PKC and IP_3, which binds to IP_3 receptor, stimulating calcium ion release from the endoplasmic reticulum. Depletion of PIP_2, PKC and increased intracellular calcium ion that joins to CaM all suppresses the activity of Kv7 channel. This pathway was tested using PLC activator and inhibitor, but no effects on membrane potential in presence of [Ca^{2+}] 5mM and R568 were observed.

Conclusion

- Stimulation of CaSR suppress Kv7 channels current and therefore decreases membrane potential and consequently increases excitability of the neuron. Blocking of CaSR has opposite effects.
- When Kv7 is absent or blocked, no effects were seen when CaSR is stimulated. This demonstrates that Kv7 and CaSR are functionally linked together
- CaSR is not linked to Kv7 through Gq pathway which involves PLC

Future works

- Explore another G-protein pathway (e.g. Gi pathway) which may functionally link CaSR and Kv7 together

References

1. Tsantoulas C and MacMahon SB. 2014. Opening paths to novel analgesics: the role of potassium channels in chronic pain. *Trends Neurosci*. 37(3): 146-158.
2. Cifuentes M, Fuentes C, Tobar N, Acevedo I, Villalobos E, Hugo E, Ben-Jonathan N, Reyes M. 2012. Calcium sensing receptor activation elevates proinflammatory factor expression in human adipose cells and adipose tissue. *Molecular and Cellular Endocrinology*. 361(1-2):24-30.
3. Orrenius S and Nicotera P. 1994. The calcium ion and cell death. *J Neural Transm Suppl*. 43:1-11.