

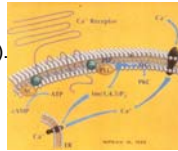
The extracellular calcium-sensing receptor: effects on epithelial (E)-cadherin and a role in cell adhesion and cell-to-cell coupling in the pancreatic islet

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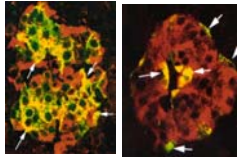
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1. Introduction

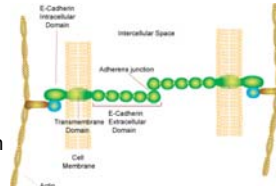
The Calcium-sensing Receptor (CaR) was first identified/cloned in the parathyroid gland (Brown *et al.*, 1993). CaR is a seven trans-membrane spanning, G-protein coupled receptor known to be involved in the regulation of systemic calcium.



The Calcium-sensing Receptor (CaR) is expressed in
Pancreatic α - and β -cells (Squires *et al.*, 2000)
Pancreatic acini (Bruce *et al.*, 1999)
Fibroblasts (McNeil *et al.*, 1998)
Neurons (Chattopadhyay *et al.*, 1998)

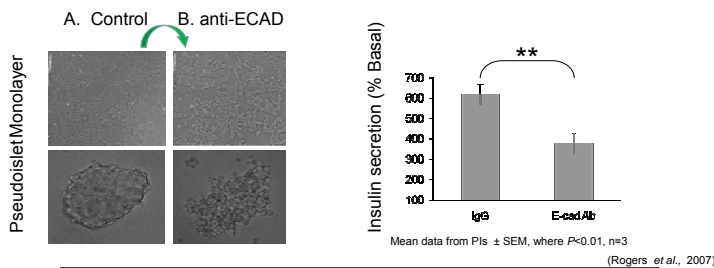


Epithelial cadherin (ECAD) is a transmembrane protein whose extracellular domain forms Calcium-dependent homodimers with cadherins expressed on adjacent cells. The cytoplasmic Domains binds to catenins which link E-cadherin to signalling proteins and the cytoskeleton.



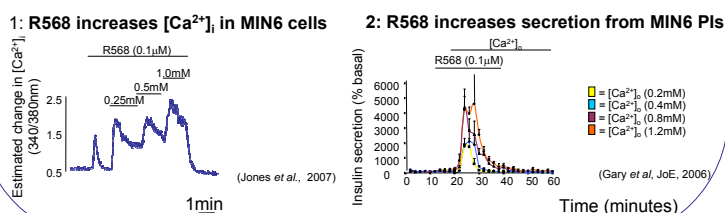
2. Background Studies - E-Cadherin regulates islet function

A: Blocking ECAD reduces cell-to-cell interactions
B: blocking ECAD reduces glucose-evoked insulin secretion



Calcimimetics can regulate islet function

Currently used in the treatment of hyperparathyroidism to reduce PTH secretion
They allosterically activate CaR by increasing the affinity of the receptor for calcium within the physiological range "Calcium sensitizers."
Calcimimetics: R568 (active version), S568 (inactive)



3. Methods

Culture of MIN6 cells and pseudoislets:

- Mouse insulinoma insulin secreting (MIN6) cells (passage 40-60) were cultured in tissue culture flasks and maintained at 37 °C in DMEM.
- MIN6 pseudoislets were grown for 6-7 days in 2% bovine gelatine (Sigma)-coated tissue culture plastic (as described previously, Hauge-Evanns *et al.*, 1999).

Extracellular calcium effect on the expression of E-cadherin :

- MIN6 cells (p47-p51) were split equally into six T25 flasks, then cultured in normal DMEM for two days followed by overnight quiescence.
- The cells were treated with different concentrations of calcium and 1 μ M of calcimimetics (A568 active and B568 inactive) and protein were collected after 6, 24 and 48 hours for assessing E-cadherin expression by western blotting.

4. Hypothesis

1. E-cadherin & the Calcium Receptor are co-regulated proteins in the islet.
2. Local calcium concentrations regulate expression of ECAD and CaR
3. Local calcium concentrations regulate intra-islet communication and function

5. Results

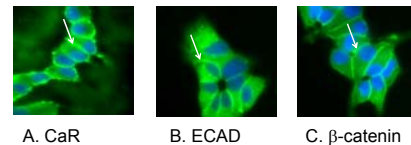


Figure 1: The CaR (A), E-cadherin (B) and β -catenin (C) are all expressed at adherens junctions in the MIN6 cells.

6. Results

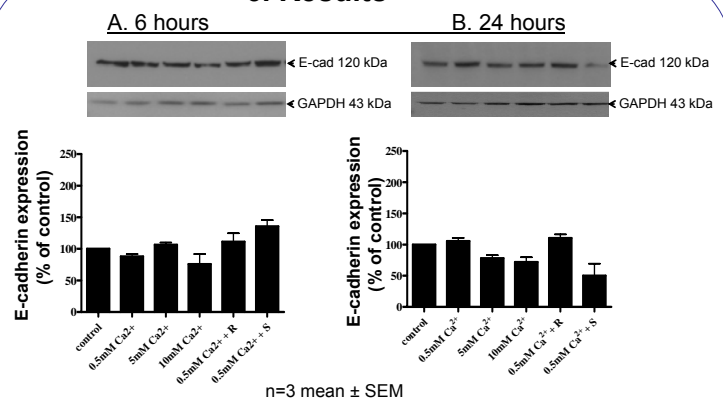


Figure 2: Incubation of MIN6 monolayers with calcium and calcimimetics shows no significant change in E-cadherin expression between 6 and 24 hours.

7. Results

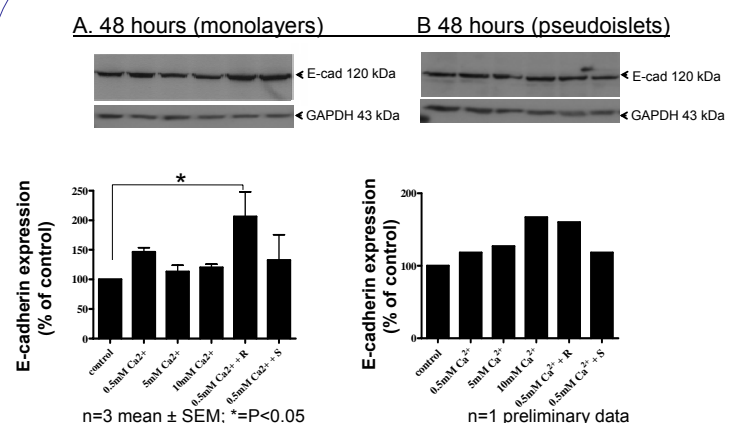


Figure 3: Activation of the CaR causes increases in E-cadherin expression in MIN6 monolayers (A) and pseudoislets (B) after 48 hours.

8. Conclusion

- Our results suggest that extracellular calcium enhance the expression of E-cadherin through activation of CaR and thereby maintain adhesion and coupling of β -cells that lead to improve the process of insulin secretion.
- Localisation of CaR, E-cadherin and β -catenin in the cell periphery area suggests that they play a role in maintaining cell-to-cell contact and islet function.
- These data together with the previous studies suggest a new role for calcimimetics in the treatment of diabetes.
- Future work will include neutralizing or knock-down E-cadherin and assess the effect on CaR expression.

Acknowledgements:

- 1- This work is supported by Diabetes UK
- 2- Mustafa Younis is a sponsored by the Libyan Government PhD programme