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Reciprocal regulation of the extracellular calcium-sensing receptor & epithelial-cadherin in pancreatic islets

Mustafa .G. Younis, Claire E. Hills, Gareth J Rogers, Paul E. Squires

School of Life Sciences, University of Warwick, Coventry, UK

1. Background:

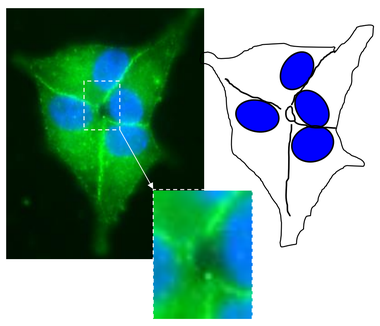

Figure 1: MIN6 cells exhibit close cell-to-cell contact. They also exhibit numerous intercellular spaces where the concentration of calcium ions, co-released with insulin, can accumulate.

•The extracellular calcium-sensing receptor (CaR) is a 7-transmembrane spanning G-protein-coupled receptor commonly associated with the regulation of systemic Ca^{2+}-homeostasis. However, it is becoming increasingly apparent that the CaR has diverse effects outside this primary role.

•In keratinocytes, inactivation of CaR expression has been shown to suppress the assembly of the cell adhesion associated epithelial-cadherin (ECAD) and β-catenin-PI3K complex [1]. Pancreatic β-cells express the CaR [2]. From our own studies, pancreatic β-cells demonstrate a close association between adherens junction proteins and secretory granules [3]. Neutralising ECAD-mediated cell adhesion decreases glucose-evoked synchronicity in Ca^{2+}-signals between adjacent cells within islets [4]. Together, these findings suggest an intriguing link between cell-cell adhesion, and CaR expression/function in pancreatic β-cells.

•In the current study, we have assessed the effect of activating the CaR via the calcimimetic R568 on the expression of ECAD, β-catenin, L-type VDCC and CaR itself. To determine if cell-to-cell contact, alters CaR expression and ultimately the β-cell function., ECAD-mediated cell-cell adhesion was reduced by immuno-neutralising ECAD. The effects on non-nutrient evoked changes in cytosolic calcium and glucose-evoked insulin secretion were examined.

2. Methods:

***Maintenance of MIN6 cells*:**

❑Mouse insulinoma insulin secreting (MIN6) cells (passage 35-45) were cultured in DMEM and maintained under standard tissue culture conditions at 37ºC and 5%CO_2.

❑MIN6 were grown for 6-7 days in 2% bovine gelatine (Sigma)-coated tissue culture plastic as described previously Hauge-Evans et al.,1999).

CaR expression in MIN6 cells:

❑Western blot analysis: Filters were analyzed with monoclonal anti-CaR antibody produced in mouse (1:250).

❑CaR immunoreactivity was assessed by fluorescence immunocytochemistry using a polyclonal anti-CaR antibody produced in rabbit (1:50)

Microfluorimetry:

❑Changes in [Ca2+]i were measured by adding fluorescent Ca2+-sensitive dye Fura-2/AM (5μM; Sigma) to MIN6 cells grown in coverslips for 30 minutes at 37 ºC and the coverslip then transferred to a steel perfusion chamber and sodium-rich balanced salt solution were added to cells using low pressure rapid perfusion system connected to the Axiovert200 Research epi-fluorescent microscope (Carl Zeiss, UK). Cells were alternatively illuminated at 340nm and 380nm using a Metafluor imaging workbench (Universal Imaging Corporation Limited, UK).

❑***Insulin secretion:***

❑MIN6 pseudoislets were treated with anti-ECAD antibody (68 μg/ml) and control cells with rat anti-IgG antibody for 24hours, treatments replaced with bicarbonate-buffered physiological salt solution containing 2mM glucose (Gey-Gey, 1936) for one hour. The insulin content of the incubation medium was assessed using ELISA (Immunodiagnostic System Limited, UK).

Data Analysis:

❑Autoradiographs were quantified by densitometry using TotalLab 2003 (NonLinear Dynamics, Durham, NC USA). Controls were normalized to 100% and data from all other experimental conditions compared to this. Statistical analysis of data was performed using either a paired t-test or a one-way ANOVA test with a Tukey's multiple comparison post-test. Data are expressed as mean±SEM where $P<0.05$ was considered significant.

3. Results:

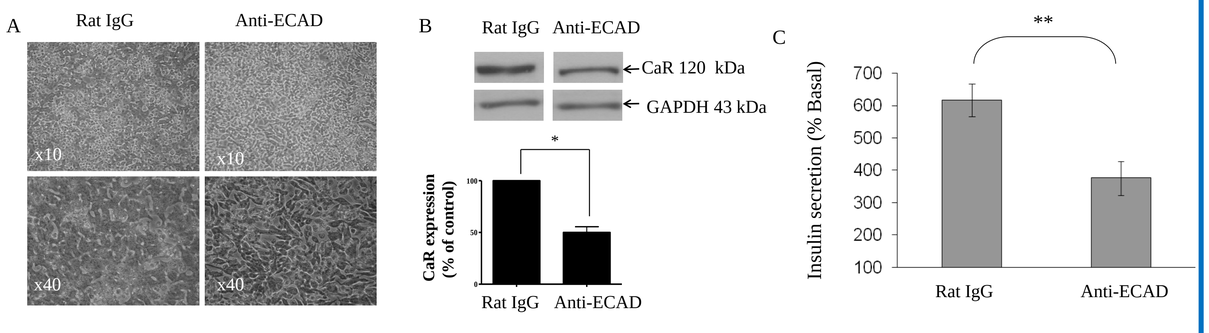

Figure 4. *Cell-to-cell contact affects CaR-expression and insulin secretion:* (A) Phase images of MIN6 cell configured as monolayers demonstrates how neutralizing E-cadherin ligation, using an anti-ECAD antibody (68μg/ml), reduces cell-to-cell contact. Western blotting analysis confirming the expression of the CaR (120kDa) and GAPDH (43kDa) from MIN6 cell lysates. Overnight incubation with (68μg/ml) immuno-neutralizing anti-ECAD (B) halves CaR-expression compared to IgG-treated control cells. The same treatment significantly reduces glucose-evoked insulin secretion (C) Data is mean ± sem, where *$P<0.05$ and **$P<0.01$, n=3.

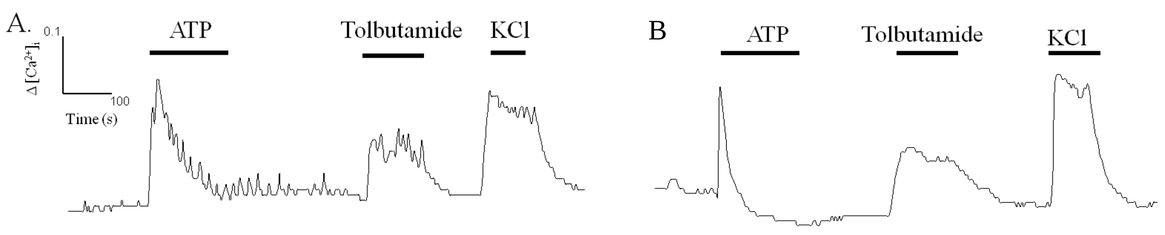

Figure 5. *Loss of E-cadherin reduces ATP-evoked Ca^{2+}-influx:* In panel A, ATP (50μM), tolbutamide (10μM) and KCl (20mM)-evoked changes in $[Ca^{2+}]_i$ in control (rat IgG-treated) cells. Following neutralization of endogenous E-cadherin ligation using the anti-ECAD immuno-neutralizing antibody (panel B), responses of comparable basal-to-peak amplitude were elicited by the agonists. However, the sustained Ca^{2+}-influx phase of the purinergic response to ATP was ablated.

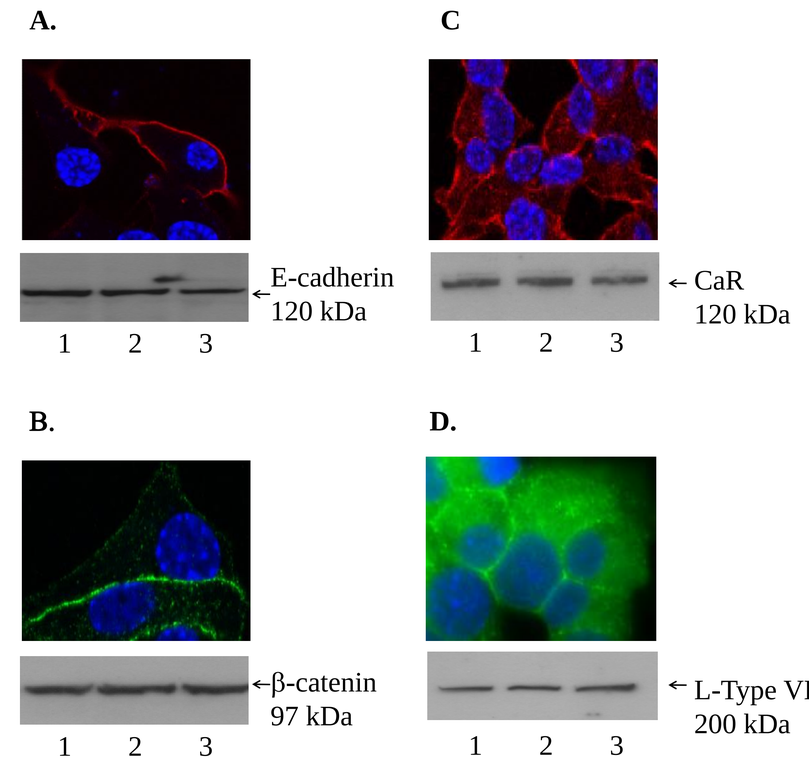

Figure 2. *Expression and localisation of CaR and adherens junction proteins:* Immunocytochemistry of monolayers and Western blot analyses of cell lysates (5μg protein/lane from 3 separate preparations) were visualised using an antibodies against E-cadherin (panel A), β-catenin (panel B), the CaR (panel C) and L-type VDCC (panel D) to confirm the presence of adhernens juction proteins and the CaR in MIN6 cells. A protein bands of approximately 120kDa (ECAD), 97kDa (β-cat), 120kDa (CaR), and 200kDa (VDCC) were detected. Controls included antibody pre-absorbed with a 10-fold excess of immunizing peptide. (Data not shown).

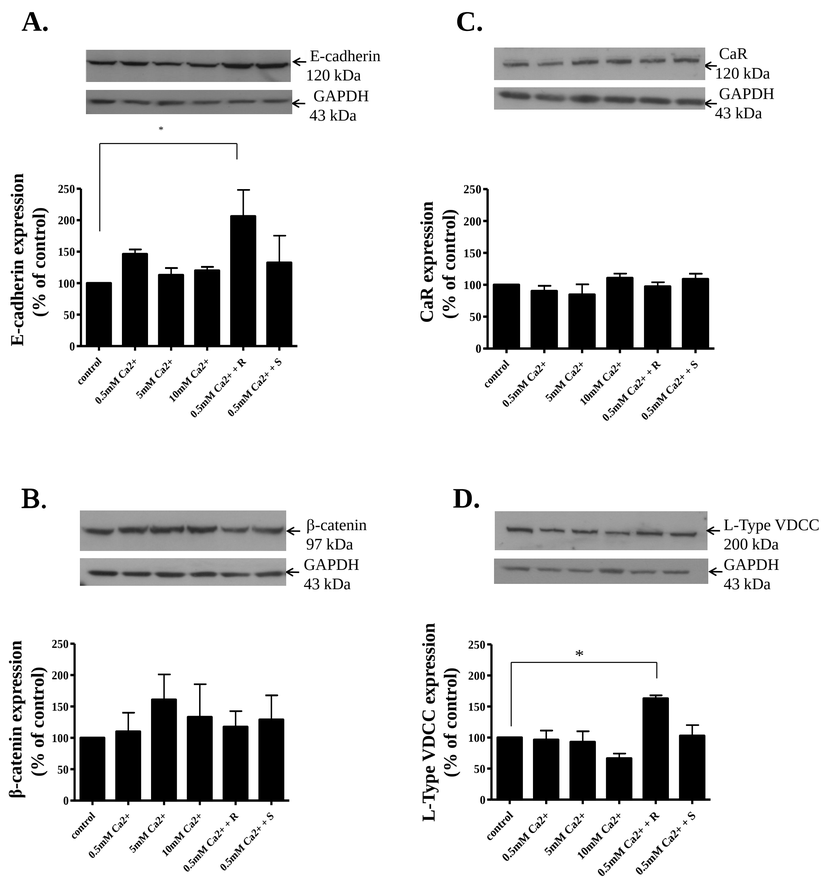

Figure 3. *CaR activation increases ECAD and VDCC expression*: Receptor specific activation of the CaR using the R stereoisomer of the calcimimetic 568 (1μM) at 0.5mM extracellular calcium, increased E-cadherin and L-type VDCC protein expression at 48hrs. The mimetic had no effect on β-catenin or CaR expression. Protein expression was unaffected by either the S-isomer or non-specific actions of high extracellular calcium. Data is mean ± sem, where *$P<0.05$, n=3.

4. Summmary and conclusions:

Neutralizing ECAD ligation changes phenotypic propertirs of MIN6 cells and reduces cell-to-cell contact which in turn results in a decrease in glucose
-evoked insulin secretion compared to normal MIN6 cells.

The sustained oscillatory component of the receptor-mediated ATP-evoced rise in [Ca2+]i was lost follwing neutralisation of ECAD ligation. This result demonstrate ECAD may be involved in in regulation of calcium entry through controling calcium channels.

The reduction in CaR expression ECAD-neutralized MIN6 cells was in accordance with increase in ECAD expression following CaR activation using calcimimetics R568, these data suggested that CaR and ECAD are reciprocally regulated in pancreatic islets.

The increased expression of L-type VDCC which resulted from CaR activation indicate that this cationic receptor could be a potential therapeutic target in the treatment of diabetes

References:

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