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COMMENTARY

The calcium-sensing receptor and insulin secretion: a role outside systemic control 15years on

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Abstract:

In the 15years since the identification and characterisation of the extracellular calcium-sensing receptor (CaR), it has become increasing apparent that this cationic binding receptor is found on many tissues, not associated with the control of plasma calcium. One of these tissues is the pancreatic islet where insulin secretion provides the basis of energy regulation. It seems inherently unlikely that the islet responds to alterations in systemic calcium and a more plausible and intriguing possibility is that the CaR mediates cell-to-cell communication through local increases in the concentration of extracellular Ca^{2+}, co-released with insulin. This short commentary explores this possibility and suggests that this novel mechanism of cell communication, along with direct coupling via gap-junctions and other local paracrine regulators helps explain why the glucose-responsiveness of the intact islet is greater than the sum of the composite parts in isolation.

Introduction:

It has been 15years since the original cloning and characterisation of the extracellular calcium-sensing receptor (CaR; Brown *et al*. 1993). Since then more than 1,000 articles have been published chronicling the role of this G-protein coupled receptor in the physiology and patho-physiology of systemic calcium regulation (extensively reviewed in Brown 2007). However, over the last decade and a half it has become apparent that the ability of cells to detect local changes in free calcium ion concentration is not restricted to tissues involved in Ca^{2+}-homeostasis. The CaR has been detected on an ever increasing range of tissue types, including oesophageal (Justinich *et al*. 2008) and colonic epithelia (Cheng *et al*. 2004), the cardiovascular system (reviewed in Smajilovic & Tfelt-Hansen 2007), hypothalamic neurons (Vizard et al. 2008), pancreatic ducts (Racz *et al*. 2002) and pancreatic α- and β-cells (Rasschaert & Malaisse 1999; Squires *et al*. 2000; Gray *et al*. 2006).

The functional significance of the CaR in tissue not involved in regulating plasma Ca^{2+} is not fully understood. In the exocrine pancreas it has been suggested that the CaR monitors extracellular Ca^{2+} in pancreatic juice to limit the risk of calcium carbonate stone formation (Bruce *et al*. 1999), and in gastrin secreting cells of the human antrum the CaR may detect dietary Ca^{2+} (Ray *et al*. 1997; Buchan *et al*. 2001). However, a more global explanation for the role of the CaR in these disparate tissues could be in its ability to detect local fluctuations in Ca^{2+}, mediating cell-to-cell communication and coupling function. Cells communicate locally via gap junctions that physically connect adjacent cells and permit the free-flow of ions and small molecules (Hills *et al*. 2006), or through the release of local paracrine messengers (Squires *et al*. 2002). Recent evidence, from our work on pancreatic β-cells, suggests an important function for the CaR in mediating cell-to-cell communication within islets to co-ordinate insulin secretory responses (Jones *et al*. 2007). Local changes in the concentration of extracellular Ca^{2+} can occur as result of changes in Ca^{2+}-influx/efflux pathways across the plasma-membrane (Green *et al*. 2007). Additionally, secretory granules contain high concentrations of calcium that is released upon exocytosis (Belan *et al*. 1998).

As the volume of space between cells is often small, large changes in Ca^{2+} concentration can occur in the micro-environment immediately surrounding cells (Perez-Armendariz & Atwater 1986). These local extracellular 'hot-spots' of calcium are sufficient to activate the CaR on neighbouring cells and facilitate cellular co-operation.

CaR: cell-to-cell communication and the pancreatic islet

Several theories have been proposed to explain the synchronous and cooperative activity of islets when compared to non-cooperative events in isolated individual β-cells including direct communication via gap junctions (Moreno *et al*, 2005; Rogers *et al*. 2007), the presence of other endocrine cells (Ishihara *et al*, 2003), as well as the existence of extracellular diffusible mediators (Squires *et al*. 2002; Hellman *et al*. 2004). The possibility that local changes in extracellular Ca^{2+} resulting from the efflux of mobilised Ca^{2+} in one cell are sufficient to activate the CaR on an adjacent cell was elegantly demonstrated in a HEK293 model system (Hofer *et al*. 2000). These studies suggested that the extrusion of Ca^{2+} from stimulated cells, recruited neighbouring cells, allowing amplification and integration of a tissue wide response (reviewed in Hofer *et al*. 2004). In the pancreas we've long argued that close cell-to-cell contact improves the functional responsiveness of cells and augments insulin secretion (Hauge-Evans *et al*. 1999). Activation of the CaR using receptor-specific calcimimetics (reviewed in Trivedi *et al*. 2008) enhances insulin secretion from human islets (Gray *et al*. 2006) and provides an obvious link by which glucose-evoked release of calcium-rich secretory granules feeds forward to synchronise secretion and perpetuate the whole islet response. The proposed model of this CaR-mediated propagation of signals across the islet is illustrated in the schematic below. Here glucose-evoked changes in insulin secretion in one cell can stimulate insulin secretion from neighbouring cells expressing the CaR, through co-release of divalent cations, ultimately improving overall secretory function.

It is unusual for receptor-mediated stimuli to initiate insulin release in the absence of stimulatory glucose concentrations. However, calcimimetic activation of the CaR in human

and rodent β-cells transiently increases insulin secretion, without the need for an associated increase in nutrient stimulation (Gray *et al*. 2006), stressing the potential importance of the CaR to islet function. It is therefore surprising that activating mutations of the CaR as seen in autosomal-dominant hypocalcaemia (extensively reviewed in Egbuna & Brown, 2008), cause hypocalcaemia of varying severity without hypoglycaemia as expected from an increase in insulin secretion under the current model. This discrepancy could be explained by the fact that hypocalcaemia has been shown to reduce insulin secretion (Schlumbohm & Harmeyer, 2002), perhaps through a reduced drive for Ca^{2+}-entry following glucose-stimulated closure of the ATP-sensitive potassium channels on the β-cells. Certainly if CaR function is increased in pancreatic β-cells from a background of eucalcemia there is an increase in insulin secretion (Grey *et al*, 2006), an effect that may form the basis of the intra-arterial calcium stimulation test for the detection of insulinomas (Kato *et al*. 1997; Won *et al*, 2003). Loss of CaR function may partially explain increased prevalence of coincident diabetes in patients presenting with primary hyperparathyroidism, where the loss of CaR-function in the parathyroid increases PTH-secretion (reviewed in Taylor & Khaleeli, 2001).

CaR: a role in cell adhesion and proliferation in the islet.

The biosynthetic and secretory function of the islet depends largely on the architecture of the islet, itself dictated by specialised cell adhesion molecules such as the cell surface adhesion protein epithelial (E)-cadherin (ECAD) and β-catenin (reviewed in D'Souza-Schorey 2005). The co-localisation of adherens junction proteins to secretory granules (Hodgkin *et al*. 2007) suggests that the adherens junction may play a novel role in β-cell function, both in terms of β-cell proliferation (Carvell *et al*. 2007) and insulin secretion (Hodgkin *et al*. 2007; Rogers *et al*. 2007). Neutralising ECAD-mediated cell adhesion decreases glucose-evoked synchronicity in Ca^{2+}-signals between adjacent cells within islets (Rogers *et al*. 2007) and evidence from human epidermal keratinocytes suggests that inactivation of the CaR suppresses the assembly of the ECAD-catenin-PI3K complex (Tu et

al. 2008). These data provide compelling evidence that the CaR influences multiple functions that ultimately regulate synchronicity of Ca^{2+}-activity between β-cells within the islet and thus dramatically impinge on insulin secretion.

Conclusion:

Calcium receptor-mediated cell-to-cell communication permits local changes in co-released Ca^{2+} to synchronise whole islet responses to secretagogues. It seems likely that the local paracrine function of extracellular Ca^{2+} acts in unison with other better characterised mechanisms for cellular coupling, to ensure appropriate glucose-responsiveness. Calcimimetic compounds that activate the CaR and block PTH-secretion have been developed to treat hyperparathyroidism, whilst calcilytic compounds potentially provide and anabolic therapy for osteoporosis (reviewed in Nemeth, 2004). However, the expression of a functional CaR within human pancreatic islets suggests that these therapies may have wider implications for tissues outside the normal targets for control of systemic calcium, and these possible contra-indications need to be fully explored. This short article demonstrates the importance of the CaR in orchestrating a synchronised whole islet response to improve secretory function.

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References:

1. Belan P, Gardner J, Gerasimenko O, Gerasimenko J, Mills CL, Petersen OH, Tepikin AV 1998 Isoproterenol evokes extracellular Ca^{2+} spikes due to secretory events in salivary gland cells. *Journal of Biological Chemistry* **273** 4106-4111.
2. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hedlger MA, Lytton J & Hebert SC 1993 Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. *Nature* **366** 575-580.
3. Brown EM 2007 The calcium-sensing receptor: physiology, pathophysiology and CaR-based therapeutics. *Subcellular Biochemistry* **45** 139-167.
4. Bruce JIE, Yang X, Ferguson CJ, Elliot AC, Steward MC, Maynard-Case R, Riccardi D 1999 Molecular and functional identification of a Ca^{2+} (polyvalent cation)-sensing receptor in rat pancreas. *Journal of Biological Chemistry* **274** 20561-20568.
5. Buchan AMJ, Squires PE, Ring M & Meloche MR 2001 Mechanism of action of the calcium-sensing receptor in human antral gastin cells. *Gastroenterology* **120** 1128-1139.
6. Carvell MJ, Marsh PJ, Persaud SJ & Jones PM 2007 E-cadherin interactions regulate β-cell proliferation in islet-like structures. *Cellular Physiology and Biochemistry* **20** 617-626.
7. Cheng SX, Giebel JP & Hebert SC 2004 Extracellular polyamines regulate fluid secretion in rat colonic crypts via the extracellular calcium-sensing receptor. *Gastroenterology* **126** 148-158.
8. D'Souza-Schorey C 2005 Disassembling adherens junctions: breaking up is hard to do. *Trends in Cell Biology* **15** 19-26.
9. Egbuna OI, Brown EM 2008 Hypercalcaemia and hypocalcaemia conditions due to calcium-sensing receptor mutations. *Best Practice and Research Clinical Rheumatology* **22** 129-148.
10. Gray E, Muller D, Squires PE, Asare-Anane H, Huang G-C, Amiel S, Persaud SJ & Jones PM 2006 Activation of the extracellular calcium-sensing receptor initiates

insulin secretion from human islets of Langerhans: involvement of protein kinases. *Journal of Endocrinology* **190** 703-710.

11. Green AK, Stratton RC, Squires PE & Simpson AWM 2007 Atrial natriuretic peptide attenuates elevations in Ca^{2+} and protects hepatocytes by stimulating net plasma membrane Ca^{2+} efflux. *Journal of Biological Chemistry* **282** 34542-34554.
12. Hauge-Evans AC, Squires PE, Persaud SJ & Jones PM 1999 Pancreatic β-cell-to-β-cell interactions are required for integrated responses to nutrient stimuli: enhanced Ca^{2+} and insulin secretory responses of MIN6 pseudoislets. *Diabetes* **48** 1402-1408.
13. Hellman B, Dansk H & Grapengiesser E 2004 Pancreatic β-cells communicate via intermittent release of ATP. *American Journal of Physiology* **286** E759-E765.
14. Hills CE, Bland R, Wheelans DC, Bennett J, Ronco PM & Squires PE 2006 Glucose-evoked alterations in connexin 43-mediated cell-to-cell communication in human collecting duct: a possible role in diabetic nephropathy. *American Journal of Physiology* **291** F1045-F1051.
15. Hodgkin MN, Rogers GJ & Squires PE 2007 Co-localization between β-catenin and insulin suggests a novel role for the adherens junction in β-cell function. *Pancreas* **34** 170-171.
16. Hofer AM, Curci S, Doble MA, Brown EM & Soybel DI 2000 Intercellular communication medicated by the extracellular cacium-sensing receptor. *Nature Cell Biology* **2** 392-398.
17. Hofer AM, Gerbino A, Caroppo R & Curci S 2004 The extracellular calcium-sensing receptor and cell-cell signalling in epithelia. *Cell Calcium* **35** 297-306.
18. Ishihara H, Maechler P, Gjinovci A, Herrera P-L & Wollheim CB 2003 Islet β-cell secretion determines glucagons release from neighbouring α-cells. *Nature Cell Biology* **5** 300-335.

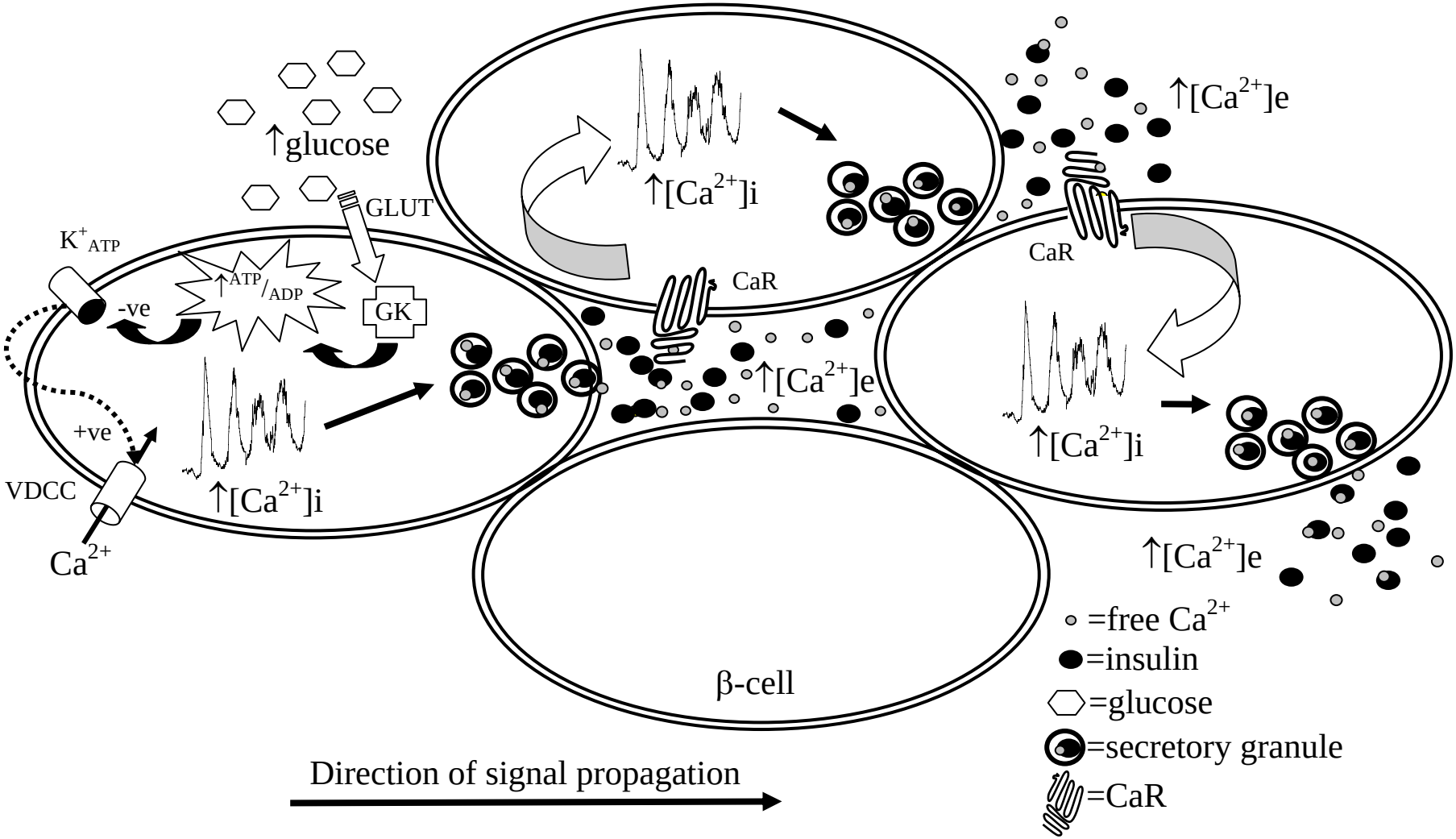
19. Jones PM, Kitsou-Mylona I, Gray E, Squires PE & Persaud SJ 2007 Expression and function of the extracellular calcium-sensing receptor in pancreatic β-cells. *Archives of Physiology and Biochemistry* **113** 98-103.
20. Justinich CJ, Mak N, Pacheco I, Mulder D, Wells RW, Blennerhassett MG & MacLeod RJ 2008 The extracellular calcium-sensing receptor (CaSR) on human esophagus and evidence of expression of the CaSR on the esophageal epithelial cell line (HET-1A). *American Journal of Physiology* **294** G120-G129.
21. Kato M, Doi R, Imamura M, Furutani M, Hosotani R & Shimada Y 1997 Calcium-evoked insulin release from insulinoma cells is mediated via calcium-sensing receptor. *Surgery* **122** 1203-1211.
22. Moreno AP, Berthould V, Perez—Palacios G & Perez-Armendariz EM 2005 Biophysical evidence that connexin-36 forms functional gap junction channels between pancreatic mouse β-cells. *American Journal of Physiology* **288** E948-E956.
23. Nemeth EF 2004 Calcimimetic and calcilytic drugs: just for parathyroid cells? *Cell Calcium* **35** 283-289.
24. Perez-Armendariz E & Atwater I 1986 Glucose-evoked changes in $[K^+]$ and $[Ca^{2+}]$ in the intercellular spaces of the mouse islet of Langerhans. *Advances in Experimental Medicine and Biology* **211** 31-51.
25. Racz GZ, Kittel A, Riccardi D, Case RM, Elliot AC & Varga G 2002 Extracellular calcium sensing receptor in human pancreatic cells. *Gut* **51** 705-711.
26. Rasschaert J & Malaisse WJ 1999 Expression of the calcium-sensing receptor in pancreatic islet β-cells. *Biochemical and Biophysical Research Communications* **264** 615-618.
27. Ray JM, Squires PE, Curtis SB, Meloche MR & Buchan AMJ 1997 Expression of the calcium-sensing receptor on human antral gastrin cells in culture. *Journal of Clinical Investigation* **99** 2328-2333.

28. Rogers GJ, Hodgkin MN & Squires PE 2007 E-cadherin and cell adhesion: a role in architecture and function in the pancreatic islet. *Cellular Physiology and Biochemistry* **20** 987-994.
29. Schlumbohm C & Harmeyer J 2002 Hypocalcemia reduces insulin turnover but not insulin-mediated glucose metabolism in piglets. *Acta Diabetologica* **39** 191-202.
30. Smajilovic S & Tfelt-Hansen J 2007 Calcium acts as a first messenger through the calcium-sensing receptor in the cardiovascular system. *Cardiovascular Research* **75** 457-467.
31. Squires PE, Harris TE, Persaud SJ, Curtis SB, Buchan AMJ, Jones PM 2000 The extracellular calcium-sensing receptor on human β-cells negatively modulates insulin secretion. *Diabetes* **49** 409-417.
32. Squires PE, Persaud SJ, Hauge-Evans AC, Gray E, Ratcliff H & Jones PM 2002 Co-ordinated Ca^{2+}-signalling within pancreatic islets: does β-cell entrainment require a secreted messenger. *Cell Calcium* **31** 209-219.
33. Taylor WH & Khaleeli AA 2001 Coincident diabetes mellitus and primary hyperparathyroidism. *Diabetes Metababolism Research and Reviews* **17** 175-180.
34. Trivedi R, Mithal A & Chattopadhyay N 2008 Recent updates on the calcium-sensing receptor as a drug target. *Current Medicinal Chemistry* **15** 178-186.
35. Tu C-L, Chang W, Xie Z & Bikle DD 2008 Inactivation of the calcium sensing receptor inhibits E-cadherin-mediated cell-cell adhesion and calcium-induced differentiation in human epidermal keratinocytes. *Journal of Biological Chemistry* **283** 3519-3528.
36. Vizard TN, O'Keeffe GW, Gutierrez H, Kos CH, Riccardi D & Davies AM 2008 Regulation of axonal and dendritic growth by the extracellular calcium-sensing receptor. *Nature Neuroscience* **11** 285-291.
37. Won JGS, Tseng H-S, Yang A-H, Yang K-T, Jap T-S, Kwok C-F, Lee CH & Lin H-D 2003 Intra-arterial calcium stimulation test for detection of insulinomas: detection

253 rate, responses of pancreatic peptides, and its relationship to differentiation of tumour
254 cells. *Metabolism* **52** 1320-1329.

Figure Legend:

CaR-mediated cell-to-cell communication within pancreatic islets: Glucose metabolism within pancreatic β-cells is limited by the low affinity glucokinase (GK). The resultant rise in ATP/ADP ratio closes the ATP-sensitive potassium channels (K^+_{ATP}), depolarising the cell membrane and opening voltage-dependant Ca^{2+}-channels (VDCC). Calcium enters the cell down a concentration gradient and stimulates insulin secretion (•). Divalent cations, including free Ca^{2+} (°) are co-released with insulin, increasing the local concentration of extracellular calcium ($\uparrow[Ca^{2+}]_e$) in the intra-islet space. These changes act in a paracrine fashion that is detected by the extracellular Ca^{2+}-sensing receptor (CaR) on adjacent cells. CaR-mediated increases in $[Ca^{2+}]_i$, propagate the signal across the islet, thus co-ordinating activity and enhancing glucose-induced insulin secretion.


↑glucose
GLUT
K^+_{ATP}
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VDCC
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CaR
↑[Ca^{2+}]i
↑[Ca^{2+}]e
β-cell
=free Ca^{2+}
=insulin
=glucose
=secretory granule
=CaR
Direction of signal propagation