COMMENTARY

Non- Ca^{2+} -homeostatic functions of the extracellular Ca^{2+} -sensing receptor (CaR) in endocrine tissues

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Abstract

The extracellular Ca^{2+} -sensing receptor (CaR) links changes in the concentration of extracellular Ca^{2+} to changes in cell function. For cells involved in the control of systemic Ca^{2+} concentration, this provides an efficient receptor-mediated mechanism to rapidly counteract slight fluctuations in the circulating concentration of Ca^{2+} . However, all cells that express the CaR are not necessarily involved in Ca^{2+} homeostasis. The recent localisation of CaR expression on a variety of cell types more usually

associated with non- Ca^{2+} -homeostatic endocrine function may have serious repercussions for the interpretation of data in those systems which routinely culture cells under standard hypercalcaemic conditions. This short commentary considers the literature surrounding the identification of the CaR and the potential effects of its localisation on endocrine cells not directly involved in the control of systemic Ca^{2+} homeostasis.

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Introduction

Changes in the concentration of intracellular calcium ($[\text{Ca}^{2+}]_i$) play a central role in a vast array of diverse cellular processes from proliferation, growth and development, to hormone secretion and neuronal transmission. These changes in $[\text{Ca}^{2+}]_i$ arise via the mobilisation of stored Ca^{2+} from cytoplasmic pools, and/or Ca^{2+} entry across the plasma membrane via various influx pathways. The concentration of systemic free ionised Ca^{2+} is maintained within a narrow range (1.1–1.3 mM) by mechanisms involving parathyroid hormone (PTH)-secreting cells, and various other specialised tissues. Because of this, it is widely perceived that the concentration of Ca^{2+} outside those cells not directly involved in systemic Ca^{2+} homeostasis merely serves to sustain normal cellular function. As a result, most researchers culture cells in standard tissue culture media, which commonly contain a slightly supra-physiological Ca^{2+} concentration (usually 1.8–2 mM, as compared with an ambient *in situ* concentration of 1.1–1.3 mM). However, it is becoming increasingly apparent that local Ca^{2+} concentrations at the cell surface of many cells vary markedly, and that these changes may influence cell physiology via a receptor-mediated mechanism.

Extracellular Ca^{2+} -sensing receptor (CaR) in the parathyroid

In 1993 Brown *et al.* cloned the extracellular CaR from bovine PTH-secreting cells. The receptor, which shares limited homology to the metabotropic glutamate receptor, consists of a large extracellular Ca^{2+} -binding region coupled to a seven trans-membrane spanning region similar to the G-protein-coupled receptor superfamily. In PTH-secreting cells, activation of the receptor via small perturbations of extracellular Ca^{2+} evokes a phospholipase C (PLC)-mediated increase in cytosolic free Ca^{2+} , via mobilisation of inositol 1,4,5-trisphosphate (InsP_3)-gated stored Ca^{2+} . Calcium-induced increases in cytosolic Ca^{2+} mediated via the CaR are likely to be sustained by Ca^{2+} influx, either through voltage-gated Ca^{2+} channels (VGCC) (Scherubi *et al.* 1991), or non-selective cation channels (NSCC) (Silva *et al.* 1994), depending on the cell type being investigated. Activation of the CaR is coupled to downstream signalling events through multiple G-protein-mediated cascades, and elevation in extracellular Ca^{2+} also inhibits agonist-evoked cAMP accumulation (Fig. 1). The net effect is reduced parathyroid hormone secretion, and the subsequent rectification of systemic normocalcaemia (extensively reviewed in Chattopadhyay *et al.* 1996, Baum & Harris 1998). It is interesting to

Figure 1 A schematic of the intracellular effector systems linked to the extracellular CaR found in various cell types. Activation of the CaR stimulates a Gq-mediated stimulation of PLC. Cytosolic Ca^{2+} is elevated via InsP_3 (IP_3)-gated Ca^{2+} mobilisation from cytosolic stores (ER), and Ca^{2+} influx via tissue-specific voltage-dependent Ca^{2+} channels (VDCC) and/or NSCC. The generation of protein kinase C (PKC) by diacylglycerol (DG) may act as a negative feedback mechanism as the CaR has several PKC phosphorylation sites. Gi-mediated inhibition of adenylate cyclase (AC) inhibits the generation of cAMP. Depending on the tissue type, receptor activation may stimulate or inhibit secretion. (Based on work by Kifor & Brown 1988, Chen *et al.* 1989, Brown *et al.* 1993, Nemeth 1995.)

consider that secretion from calcitonin-secreting C-cells of the thyroid is stimulated by elevated systemic Ca^{2+} , but both PTH-secreting cells and C-cells reportedly express the same CaR (Garrett *et al.* 1995). The clinical significance of CaR expression in PTH-secreting cells is highlighted by the marked pathophysiology observed in patients with altered receptor affinity for extracellular Ca^{2+} (Pollak *et al.* 1994a, for review see Pearce & Thakker 1997, Thakker 1998). Variations in receptor affinity can arise from a single point mutation in the CaR, as seen in

familial hypocalciuric hypercalcaemia (Pollak *et al.* 1994b, Chou *et al.* 1995).

Other CaR agonists

Although Ca^{2+} is the most relevant physiological ligand, other polyvalent cations and polycationic molecules, e.g. spermine, spermidine, neomycin and gadolinium, also activate the CaR (Brown *et al.* 1993, Quinn *et al.* 1997).

Table 1 Localisation of CaR expression

	Type/cell type	Species	Reference
Tissue involved in Ca²⁺ homeostasis	PTH cell	Human, bovine, rodent	Brown <i>et al.</i> (1993); Garrett <i>et al.</i> (1995)
	C-cells	Human, rodent, sheep	Garrett <i>et al.</i> (1995)
	Kidney	Rodent	Riccardi <i>et al.</i> (1996, 1998)
	Intestine	Rodent	Chattopadhyay <i>et al.</i> (1998); Gama <i>et al.</i> (1997)
Tissue not involved in Ca²⁺ homeostasis	Brain	Rodent	Raut <i>et al.</i> (1996); Rogers <i>et al.</i> (1997)
	G-cells	Human	Ray <i>et al.</i> (1997)
	Pancreas	Human, rodent	Squires <i>et al.</i> (1999); Wang <i>et al.</i> (1995)
	Lens cells	Human	Chattopadhyay <i>et al.</i> (1997a)
	Keratinocytes	Human	Bikle <i>et al.</i> (1996)
	Squamous carcinoma	Human	Wakita <i>et al.</i> (1997)
	Fibroblasts	Rat	McNeil <i>et al.</i> (1998)
	Breast	Human	Cheng <i>et al.</i> (1998)

These polycationic CaR agonists probably interact with the CaR electrostatically, screening charged amino acids on the extracellular domain of the receptor (Quinn *et al.* 1998). Activation of the CaR, via these polyvalent ligands, may be subject to modification by conditions such as a change in ionic strength (Traynelis *et al.* 1993). The capacity of the CaR to sense ionic strength (and therefore sodium concentration), and its presence in areas of the hypothalamus that mediate vasopressin secretion (Rogers *et al.* 1997) indicate that CaR has a role in transducing the effects of changes in ionic composition, with implications for the neural control of fluid balance (Washburn *et al.* 1999).

CaR in gastrin-secreting G-cells

Although there are now extensive data on the role of the CaR in systemic Ca²⁺ homeostasis, it has become increasingly apparent that CaR expression is not limited only to cells directly involved in the maintenance of normocalcaemia (Chattopadhyay *et al.* 1997b) (see Table 1). The CaR has been localised to cells of the central nervous system (Rogers *et al.* 1997), lens cells (Chattopadhyay *et al.* 1997a), the intestine (Gama *et al.* 1997, Ray *et al.* 1997, Chattopadhyay *et al.* 1998) and the pancreas (Silva *et al.* 1994, Wang *et al.* 1995, Kato *et al.* 1997). The precise function of the receptor in these diverse tissues is unclear.

Gastrin secreting G-cells of the human antrum exhibit concentration-dependent, sustained and reversible Ca²⁺-induced changes in free cytosolic [Ca²⁺]_i and gastrin (Ray *et al.* 1997). However, the Ca²⁺ concentration required to activate these effects maximally is substantially higher than those required to activate the receptor in the parathyroid (3.6 mM compared with 1.1–1.3 mM). This decreased affinity of the receptor to its endogenous agonists in the

antrum of the stomach is consistent with the concentrations of Ca²⁺ normally present at this site. The affinity mirrors that observed in osteoclasts which, due to their role in bone resorption, are subject to chronically high concentrations of ionised Ca²⁺ (Zaidi *et al.* 1993). However, it should be noted that, although the cation-sensing mechanism present in osteoclasts is functionally similar to the CaR, it is distinct from the CaR at the molecular level (Quarles *et al.* 1997). Although several studies have suggested that gastrin may be involved in the control of systemic Ca²⁺ concentrations (Persson *et al.* 1989, Hakanson *et al.* 1990), it is more likely that the CaR modulates signalling events elicited by other receptor agonists (Squires *et al.* 1999). Interestingly, hypercalcaemia in dogs has no effect on gastrin release (Reeder *et al.* 1970) and, in both dog and rat, hypercalcaemia results in decreased acid secretion (Barreras 1973). Humans, monkeys, cats and ferrets, however, all show elevated gastrin secretion in response to raised extracellular Ca²⁺ (Barreras & Donaldson 1967). This may reflect species differences in the tissue-specific expression of the CaR (Wada *et al.* 1999).

CaR in insulin-secreting β-cells

The primary regulator of insulin release is a change in the plasma glucose concentration. Metabolism of glucose within the β-cell alters the ATP/ADP ratio and closes ATP-dependent K⁺ channels, which depolarises the cell membrane and opens VGCCs, elevating [Ca²⁺]_i and evoking pulses of insulin release. Each voltage-dependent spike in membrane potential permits Ca²⁺ entry across the plasma membrane and links glucose metabolism with changes in [Ca²⁺]_i. Intuitively, increases in extracellular Ca²⁺ will increase the concentration gradient in favour of Ca²⁺ influx, accentuating insulin secretion. However,

several studies have observed that Ca^{2+} -induced increases in insulin secretion are transitory (Devis *et al.* 1975, Nilsson *et al.* 1987), whilst others have reported an actual decrease in secretion in response to elevated extracellular Ca^{2+} under basal (Hellman 1975) and glucose-stimulated conditions (Hales & Milner 1968, reviewed in Wollheim & Sharp 1981). The recent localisation of the CaR on rodent (Rasschaert *et al.* 1998) and human pancreatic β -cells (Squires *et al.* 2000) may help to explain these counter-intuitive data. Extracellular Ca^{2+} is essential for glucose-induced insulin secretion (Hellman 1975), and increasing extracellular Ca^{2+} from 1.3 to 5 mM increases insulin secretion from human pancreatic islets (Squires *et al.* 2000). However, this increase is transient, with the predominant effect being a sustained, reversible inhibition of both basal and glucose-induced insulin secretion. This Ca^{2+} -induced inhibition of secretion is reminiscent of that observed in the PTH-secreting cells; however, changes in $[\text{Ca}^{2+}]_i$ were transient and cAMP content increased, suggesting that the PLC/InsP₃ and the adenylate cyclase/cAMP pathways are not responsible for the prolonged inhibitory component of the response. An alternative, unidentified, pertussis toxin-sensitive G-protein-coupled mechanism, distal to the initial activation of the receptor (Sharp 1996), may mediate the observed inhibition of insulin secretion by elevated extracellular Ca^{2+} , in spite of associated increases in $[\text{Ca}^{2+}]_i$.

Conclusion

The preceding commentary demonstrates the functional variability of the extracellular CaR beyond the control of systemic Ca^{2+} homeostasis. Changes in extracellular Ca^{2+} no longer merely represent a passive shift in the concentration gradient, by which the extent and duration of the response to other Ca^{2+} -dependent receptor-mediated agonists are modulated. Extracellular Ca^{2+} is itself an endogenous first messenger which can alter endocrine cell function. Interestingly, the same receptor appears able to stimulate and inhibit secretion in different cell types. Considering that the majority of studies on cell lines or isolated primary cellular material culture cells at 1.8 mM basal Ca^{2+} , a concentration known to stimulate the CaR, the presence of the receptor on systems not directly involved in the maintenance of normocalcaemia has important repercussions. Clinically, the recent use of agents that mimic or potentiate the effects of extracellular Ca^{2+} on the CaR (calcimimetics) (Silverberg *et al.* 1997, Antonsen *et al.* 1998) makes the identification of secondary effects on tissues not directly involved in Ca^{2+} homeostasis of critical importance.

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