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Calcium sensing by human medullary thyroid carcinoma cells

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Abstract

Regulation of the cytoplasmic calcium concentration ($[Ca^{2+}]_i$) was studied in fura-2-loaded C-cells from two human medullary thyroid carcinomas (MTC). K^+ depolarization induced sustained rise of $[Ca^{2+}]_i$ reversed by verapamil. Elevation of external Ca^{2+} from 0.5 to 3.0 mM triggered regular oscillations or steady-state increases of $[Ca^{2+}]_i$. In Ca^{2+}-deficient medium Sr^{2+} caused steady-state increase or oscillations of the 340/380 nm fluorescence ratio. The Ca^{2+} and Sr^{2+} actions were partially reversible by verapamil. La^{3+} and Ce^{3+} elicited transient $[Ca^{2+}]_i$ peaks independent of external Ca^{2+}, but no oscillations. The results indicate that human MTC cells express a parathyroid-like Ca^{2+} sensor coupled to intracellular mobilization and influx of Ca^{2+}. A voltage-dependent Ca^{2+} influx may be of importance for the oscillations of $[Ca^{2+}]_i$.

Key words: Calcium; Calcitonin; Thyroid neoplasm; Signal transduction

1. Introduction

Extracellular Ca^{2+} regulates the secretion of calcitonin (CT) from C-cells of the thyroid gland. Elevation of external Ca^{2+} raises $[Ca^{2+}]_i$ and stimulates CT release [1,2]. However, the mechanisms by which the C-cells sense the extracellular Ca^{2+} concentration and the transduction of this signal are unknown. Using cell lines derived from rat medullary thyroid carcinoma it was recently shown that the Ca^{2+} induced increase of $[Ca^{2+}]_i$ involve oscillations [3,4]. Such oscillations are known to play an important role in the regulation of secretion from a number of different cell types [5]. The present study utilizes microfluorometry and image analysis to investigate cation induced $[Ca^{2+}]_i$ responses of single human medullary thyroid carcinoma cells loaded with fura-2.

2. Materials and methods

2.1. Chemicals

Reagents of analytical grade and deionized water were used. Sigma (St Louis, MO, USA) provided collagenase, DNase and BSA. Fura-2 acetoxymethylester (fura-2/AM) was from Calbiochem (La Jolla, CA, USA) and Ham's F-10, RPMI 1640, FBS, streptomycin, penicillin and L-glutamine from Flow (Irvine, UK), while Pharmacia Biotech (Uppsala, Sweden) provided Percoll.

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Abbreviations: CT, calcitonin; $[Ca^{2+}]_i$, cytoplasmic calcium concentration; MTC, medullary thyroid carcinoma; BSA, bovine serum albumin; FBS, foetal bovine serum.

2.2. Cell preparation

C-cells were obtained from primary MTC tumors during surgery on two patients with excessively elevated serum calcitonin levels. The specimens were minced with scissors in HEPES-buffered Ham's F-10 with 1.25 mM Ca^{2+}. A cell suspension was prepared by enzymatic digestion for 60 min at 37°C in the F-10 medium containing 1 mg/ml collagenase, 0.05 mg/ml DNase, 1.25 mM Ca^{2+} and 1.5% BSA [6]. The cell suspension was filtered through a nylon mesh (125 μm) and exposed briefly to a Ca^{2+}-deficient medium containing 1 mM EGTA. Removal of dead cells and debris was achieved by centrifugation through a 25% standard isotonic Percoll gradient. Cell viability as determined by Trypan blue exclusion exceeded 95%. Cells were cultured for 24–48 h on 22 mm circular microscopic cover glasses in RPMI 1640 medium with 1.25 mM Ca^{2+} and supplemented with 10% FBS, 50 μg/ml streptomycin, 50 IU/ml penicillin, 290 μg/ml L-glutamine.

2.3 Measurements of $[Ca^{2+}]_i$

Cells attached to the coverglasses were loaded for 30 min at 37°C with 1.0 μM fura-2/AM and rinsed. Loading and fluorescence measurements were performed in buffer containing 25 mM HEPES (pH 7.4), 3 mM glucose, 0.1% BSA, 125 mM Na^+, 5.9 mM K^+, 0.5 mM Mg^{2+} and 0.5 mM Ca^{2+} and with Cl^- as the sole anion. Additions of Sr^{2+}, K^+, La^{3+} and Ce^{3+} to the perifusion medium were done as chloride salts. The cover glasses were used as the bottom of an open chamber designed for microscopic work. The chamber wall was a broad silicon rubber ring, 9 mm inner diameter, pressed to the cover glass by the threaded chamber mount and a thin stainless steel ring. Cannulas fixed to this ring were connected to a two-channel peristaltic pump, allowing perifusion of a 2.5 mm medium layer at a rate of 1.0 ml/min. The chamber was placed on the stage of an inverted microscope within a climate box maintained at 37°C by an air-stream incubator. The microscope was equipped for epifluorescence microfluorometry with a 100× UV-fluorite objective (Nikon). A 75 W xenon arc lamp combined with 10–13 nm half band-width (HBW) interference filters in an air-turbine time-sharing multichannel spectrofluorometer provided 1 ms excitation light flashes at 340 and 380 nm every 10 ms. Emission was measured with a photomultiplier at 510 nm using a 30 nm HBW filter [7]. In addition a Magiscan system (Applied Imaging, Gateshead, UK) equipped with a filter changer providing a 340/380 nm image pair every 4.5 s was used for image analysis of $[Ca^{2+}]_i$ [8]. Images were collected through a 30 nm HBW filter at 510 nm with an intensified CCD camera (Extended ISIS-M; Photonic Science, Robertsbridge, UK). The 340/380 nm ratio

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frames were calculated after subtraction of background frames using the Tardis program (Applied Imaging). The 340/380 nm fluorescence excitation ratios was used to calculate $[Ca^{2+}]_i$ using a K_d of 224, as previously described [9]. Results are presented as mean ± S.E.

3. Results

In presence of 0.5 mM Ca^{2+} basal $[Ca^{2+}]_i$ of the MTC cells was stable at 56 ± 3.4 nM (n = 39). When elevating Ca^{2+} from 0.5 to 3.0 mM the cells responded with rhythmic oscillations of $[Ca^{2+}]_i$ (Figs. 1–3). The oscillations, which had amplitudes of 122 ± 9.4 nM (n = 27), were evident in 9 out of 12 and 18 out of 27 examined cells from the two patients. Generally, the first peak was most prominent, and the ensuing oscillations regular, but some cells displayed more complex and irregular patterns. The non-oscillating cells either exhibited a single $[Ca^{2+}]_i$ peak or a stable steady-state increase. Oscillatiory frequencies were notably different between the two patients, and averaged 1.97 ± 0.21 and 0.31 ± 0.024 per minute, respectively. Addition of 0.1 mM verapamil in presence of 3.0 mM external Ca^{2+} abolished or markedly reduced the amplitude of the oscillations (Fig. 1), and they were eliminated by lowering of external Ca^{2+} to 0.5 mM (Figs. 2 and 3).

Introduction of 5–20 mM Sr^{2+} in a Ca^{2+}-deficient medium elicited a transient increase followed by oscillations or steady-state elevations of the 340/380 nm flourescence ratio (Fig. 2). Also the Sr^{2+}-induced increase in fluorescence ratio exhibited sensitivity to verapamil.

Depolarization with 30 mM K^+ caused sustained increase in $[Ca^{2+}]_i$, but no oscillations, and this effect was reversed by verapamil (Fig. 3A). When MTC cells were challenged with 0.2 mM La^{3+} in presence of 0.5 mM Ca^{2+}, or with 0.2 mM Ce^{3+} in a Ca^{2+}-deficient medium, there were rapid $[Ca^{2+}]_i$ transients followed by return to basal levels (Fig. 3B,C). Combination of La^{3+} with 3 mM Ca^{2+} resulted in a sustained rise of $[Ca^{2+}]_i$ which was partially reversed by verapamil (Fig. 3B).

4. Discussion

C-cells respond to small elevations of external Ca^{2+} with rise of $[Ca^{2+}]_i$ and release of the Ca^{2+} lowering hormone CT [1]. Due to difficulties in obtaining sufficient amounts of tissue, little is known about regulation of $[Ca^{2+}]_i$ and CT release in human C-cells. The human MTC cell line TT does not exhibit any changes in either $[Ca^{2+}]_i$ or secretion in response to elevation of external Ca^{2+} [10]. Signal transduction may be defective in these cells, since treatment with the ionophore ionomycin raised $[Ca^{2+}]_i$ and stimulated CT release, and since electropermeabilization made the secretion senstive to external Ca^{2+}.

It is apparent from the present study that the C-cells isolated from the two patients with MTC have voltage-dependent Ca^{2+} channels. Depolarization with K^+ raised $[Ca^{2+}]_i$ and this effect was counteracted by verapamil. These findings harmonize with observations on the rat MTC 44–2 and 6–23 cell lines [1,11]. Moreover, agonists and antagonists of voltage-dependent Ca^{2+} channels

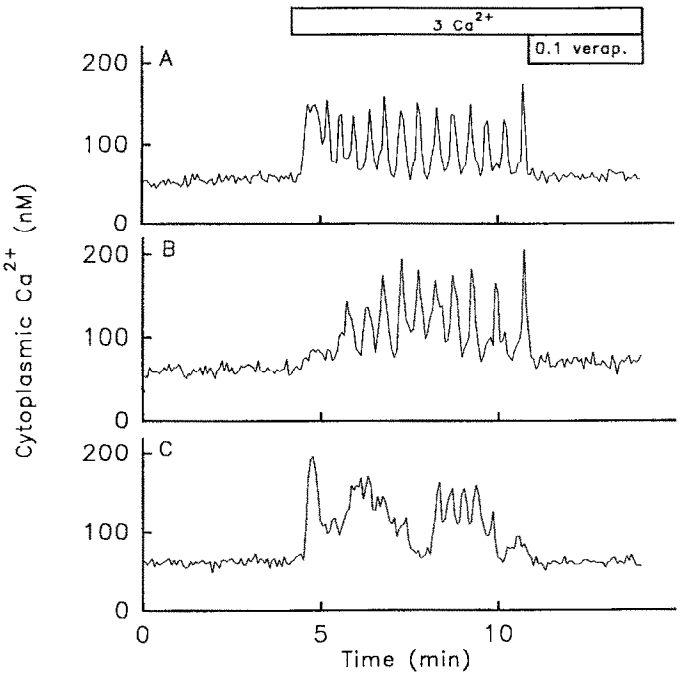

Fig. 1. Digital image analysis of $[Ca^{2+}]_i$ responses of three fura-2 loaded human MTC cells within the same image field. The cells were challenged with the indicated mM concentrations of Ca^{2+} and verapamil. The initial concentration of Ca^{2+} in the perifusion medium was 0.5 mM.

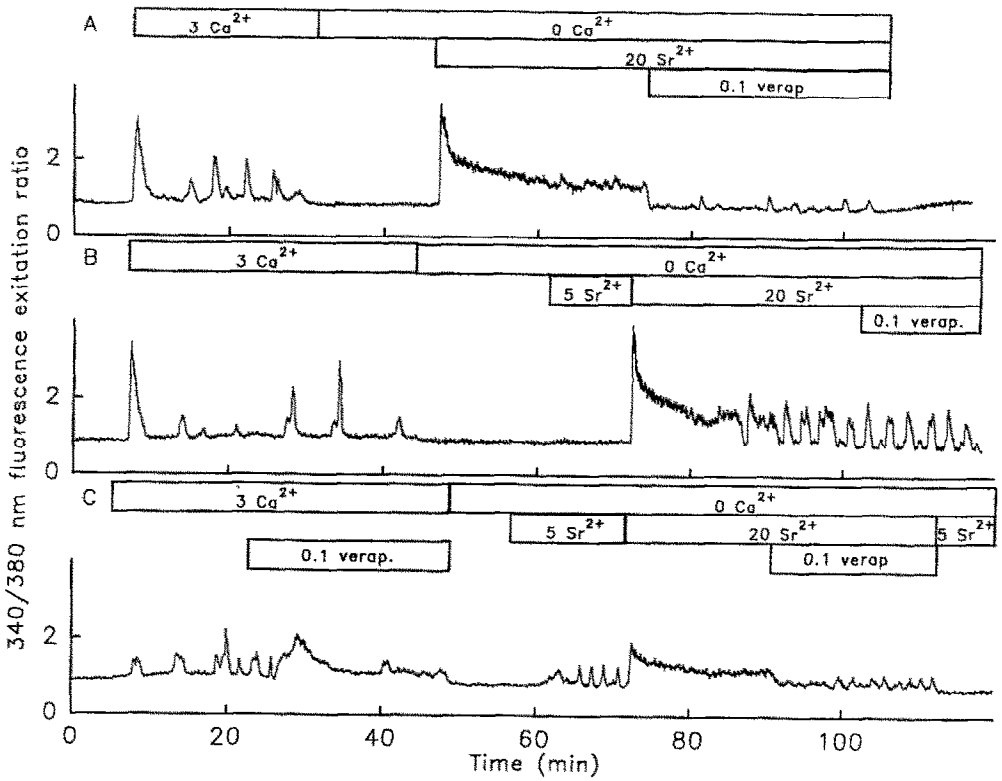

Fig. 2. Effects of Sr^{2+} and verapamil on the 340/380 nm fluorescence excitation ratio of fura-2 loaded human MTC cells. Unless otherwise indicated the Ca^{2+} concentration of the perifusion medium was 0.5 mM. The cells were challenged with the indicated mM concentrations of Ca^{2+}, Sr^{2+} and verapamil.

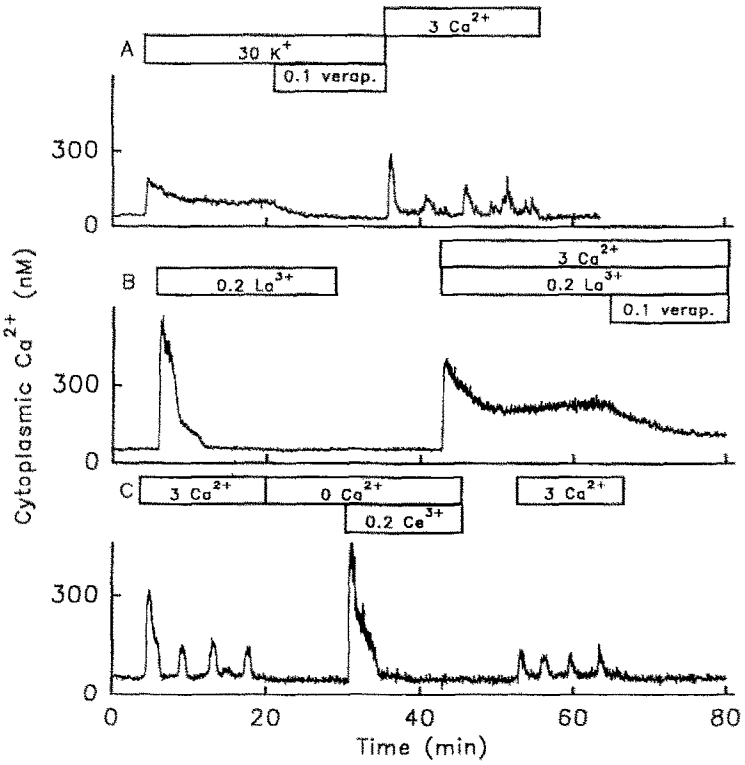

Fig. 3. Effects of K^+, La^{3+} and Ce^{3+} on $[Ca^{2+}]_i$ of fura-2 loaded MTC cells monitored by fluorescence ratio microfluorometry. Unless otherwise indicated the Ca^{2+} and K^+ concentration of the perifusion medium were 0.5 and 5.9 mM, respectively. The cells were challenged with the indicated mM concentrations of K^+, Ca^{2+}, La^{3+}, Ce^{3+} and verapamil.

stimulate and reduce CT secretion, respectively, from rat thyroparathyroid glands [12] and MTC 6–23 cells [2,13]. Elevation of external Ca^{2+} was now found to induce a $[Ca^{2+}]_i$ response sensitive to verapamil also in freshly prepared human MTC cells. However, verapamil failed to completely extinguish this response, and it could be speculated also that non-voltage-dependent mechanisms participate in regulation of $[Ca^{2+}]_i$ by external Ca^{2+}.

Like the C-cells the parathyroid parenchyma secretes a Ca^{2+} regulating hormone upon alterations in the extracellular Ca^{2+} concentration. The present data provide strong evidence indicating that a parathyroid-like Ca^{2+} sensor is present also on human MTC cells. As in the parathyroid a $[Ca^{2+}]_i$ response could be elicited by lanthanides [11], and La^{3+} has been shown to be restricted to the extracellular space [14]. La^{3+} and Ce^{3+} thus had similar effects, although the former increase and the latter quenches fluorescence of the Ca^{2+} indicator fura-2 [15]. Moreover, the earliest detectable effects of the lanthanides in parathyroid [11] and C-cells was mobilization of intracellular Ca^{2+} independent of the presence of Ca^{2+} in the external medium. Another similarity with the parathyroid is the observation that divalent cations other than Ca^{2+} can activate their own influx [16]. This mechanism explains how Sr^{2+}, which reacts with fura-2 in a Ca^{2+}-like manner [17,18], induced oscillatory and sustained increases in the 340/380 nm fluorescence ratio in the absence of external Ca^{2+}, whereas La^{3+} and Ce^{3+} only triggered a monophasic release of intracellular Ca^{2+} under similar conditions.

Although the parathyroid cells lack voltage-dependent Ca^{2+} channels [19], Ca^{2+} sensing involves cellular depolarization [20,21]. It has been proposed that the depolarization actually is carried by an inward Ca^{2+} current [22]. In view of the partial resistance of the Ca^{2+} and Sr^{2+} responses to verapamil, it seems likely that there is a similar non-voltage-dependent entry of divalent cations in the human MTC cells. Regulation of $[Ca^{2+}]_i$ and secretion in C-cells may consequently involve intracellular release of Ca^{2+}, and a depolarizing Ca^{2+} current through non-voltage-dependent channels participating in the activation of voltage-dependent Ca^{2+} entry.

The similarity in Ca^{2+} sensing between parathyroid and C-cells makes it tempting to speculate that the presence of voltage-dependent Ca^{2+} channels only in the C-cells is a determinant for their oscillatory regulation of $[Ca^{2+}]_i$. Elevation of external Ca^{2+} has been found to induce $[Ca^{2+}]_i$ oscillations also in the rat C-cell lines MTC 6–23 and 44–2 [3,4], but the former cells required pretreatment with glucagon or 8-bromo-cAMP to uncover the oscillations. Unlike the MTC 44–2 cells [4], the cells from the present two patients exhibited $[Ca^{2+}]_i$ oscillations only during exposure to elevated external Ca^{2+}. It could be speculated that the spontaneous oscillatory activity in the MTC 44–2 cells also in low external Ca^{2+} might represent functional dedifferentiation, since the secretion of Ca^{2+}-lowering CT should be turned off in a hypocalcemic environment.

Pulsatile release of hormones is considered important for preventing down-regulation of peripheral receptors [23]. Also Ca^{2+} homeostasis has been proposed to be regulated by rhythmic variations in parathyroid hormone, CT and vitamin D [24]. Fasting rats display a circadian pattern of CT secretion with two peaks per 24 h [25], but knowledge is lacking about the periodicity of CT secretion during time ranges of hours or shorter. Pulsatile secretion of PTH during short periods is well documented in human subjects with frequency components ranging from 1–6 oscillations/h [26,27]. However, it is unclear at which level this pulsatility is regulated, since isolated parathyroid cells exhibit no apparent oscillations in either $[Ca^{2+}]_i$ or secretion [4,28]. In view of the presently observed $[Ca^{2+}]_i$ oscillations during steady rise of external Ca^{2+}, it is easy to envisage a pacemaker function of the human C-cells in a rhythmically regulated Ca^{2+} homeostasis.

The oscillatory frequency was similar in different cells from each of the present patients, but varied considerably between the two tumors. The frequencies are in the same order as the <1 to 12 oscillations per minute observed in the rat MTC 44–2 and 62–3 cell lines [3,4]. If the frequencies are similar for pulsatile release of different Ca^{2+} regulating hormones, those presently observed are higher than expected from the available data on pulsatile release of PTH. Such a discrepancy may be due to dedifferentiation or to loss of coordination upon removing the cells from their natural environment.

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