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The Effect of Rottlerin in Calcium Regulation in HMC-1^{560} Cells Is Mediated by a PKC-δ Independent Effect

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ABSTRACT

The human mast cell line (HMC-1^{560}) is a good model for Ca^{2+} signaling studies, because intracellular alkalinization is the mainly histamine release stimulus without changes in the intracellular Ca^{2+} levels. This fact allows us to study Ca^{2+} changes without degranulation, since this process can affected cellular viability. Ionomycin and thapsigargin have been fully used for induced Ca^{2+} influx across SOC channels. When HMC-1^{560} cells are incubated with rottlerin, 5 μM, for 5 min a strong inhibition of ionomycin-induced Ca^{2+} influx is observed. However, when thapsigargin stimulates Ca^{2+} influx, rottlerin did not show any effect on Ca^{2+} levels. This fact point two possibilities, ionomycin and thapsigargin might activate different SOC channels or that these drugs might activate the same channel but in a different way in HMC-1^{560} cells. The rottlerin inhibition of ionomycin-induced Ca^{2+} influx is PKC-δ independent and this effect is not related with the store depletion, since rottlerin has the same effect when it is added before or after the stores are empty. FCCP, a know uncoupler of oxidative phosphorylation in mitochondria, induces the same inhibition in ionomycin Ca^{2+} influx than rottlerin which point to the mitochondria as a cellular target to rottlerin. J. Cell. Biochem. 105: 255–261, 2008.

KEY WORDS: MAST CELLS; ROTTLERIN; CALCIUM; MITOCHONDRIA; CALCIUM RESERVOIR

Mast cells are non-excitable cells that are mainly involved in inflammatory process and the immune response [Galli and Wershil, 1996]. The two main human mast cell lines used to study mast cell biology are the human mast cell line (HMC-1 line) [Butterfield et al., 1988] and the LAD ½ line (Laboratory of Allergic Diseases) [Kirshenbaum et al., 2003]. Due to their fast growth, HMC-1 cells are the most commonly studied and used in the past years.

In previous works it has been reported that in HMC-1^{560} cells, the NH_4Cl-induced intracellular alkalinization is the main stimulus to histamine release and the activation is not accompanied by intracellular calcium changes [Pernas-Sueiras et al., 2005]. HMC-1 cells are a suitable model to study intracellular calcium signaling without inducing degranulation or exocytosis processes that can affect cell viability.

The phenomenon of calcium release activated calcium current (CRAC current or I_{CRAC}) was reported in mast cells for the first time some years ago [Hoth and Penner, 1992]. The endoplasmic reticulum is an intracellular pool of calcium that has an important role in the activation of calcium influx by plasma membrane channels, known as store-operated calcium channels (SOC channels). Their activation induced the CRAC current or I_{CRAC}, a non-voltage activated inwardly rectifying current, and it is remarkably selective for calcium [Zweifach and Lewis, 1993; Parekh and Penner, 1997].

Several general models have been proposed to explain the activation process of store operated calcium influx. The first hypothesis is a model of a diffusible second messenger that link store depletion to CRAC channel activation. The second is the conformational coupling hypothesis in which the endoplasmic reticulum empty is coupled to the plasma membrane by an inositol triphosphate (IP3) receptor. The last one is the exocytosis model, where upon depletion of stores, the SOCS channels fusion with the plasma membrane via an exocytosis process [Vig and Kinet, 2007].

A controversial question about Ca^{2+} homeostasis is the type of SOC channels and how they are regulated. With regard to this, in the last years two keys players have been identified. The store Ca^{2+}

Abbreviations used: HMC-1, human mast cell line; PKC, protein kinase C; Ca^{2+}, calcium.

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sensor, STIM1, and the pore-forming CRAC channels subunit CRACM1 (or Orai1) [Vig and Kinet, 2007]. STIM1 is a transmembrane protein of the reticulum endoplasmic with a molecular weight of 77 kDa, that was identified as the crucial calcium sensor coupling the process of store depletion with calcium influx through the CRAC channels [Liou et al., 2005; Roos et al., 2005]. CRACM1 was identified as the calcium-release-activated calcium channels in the plasma membrane, an important molecular component for SOC channels and I_{CRAC} control [Feske et al., 2006; Vig et al., 2006]. However three mammalian homologous are widely expressed at the mRNA level, CRACM1, CRACM2, and CRACM3 and they all can form heteromeric channel complexes. They have distinct properties, selectivity, pharmacology regulation and feedback regulation by intracellular Ca^{2+} [Lis et al., 2007], but CRACM2 and CRACM3 are not as well characterized as CRACM1.

Another important piece of information in the activation and regulation of SOC channels seems to be the mitochondria. Changes in mitochondrial calcium dynamics have numerous effects on cell physiology. Depolarization of mitochondria prevents or inhibits I_{CRAC} [Gilabert et al., 2001]. But how depolarized mitochondria inhibits CRAC current it is still unknown.

In an earlier work [Pernas-Sueiras et al., 2006a], we reported the striking result of a total ablation of ionomycin-induced calcium increase in HMC-1^{560} caused by rottlerin, but this mechanistic effect remains to be explained. In the present study, the rottlerin effects in HMC-1^{560} calcium signaling, and the possible differences between ionomycin and thapsigargin-induced calcium entry through SOC channels were investigated. In this work the depletion of Ca^{2+} stores was evoked by two methods, application of the Ca^{2+}-ionophore Ionomycin to do permeable the ER membrane and by inhibiting the Ca^{2+}-ATPase (SERCA) with thapsigargin, a tumor-promoting sesquiterpene lactone.

Rottlerin (or mallotoxin) has been widely used as a specific modulator of PKC-δ isoform (IC50 = 3–6 μM), and also inhibits with 5- to 10-fold less potency PKC-α and PKC-β, the CAM kinase III is inhibited at 3–6 μM [Gschwendt et al., 1994]. In the past years some problems appeared concerning rottlerin effects. Rottlerin failed to show any PKC activity against the α and δ PKC isoforms, but it was reported as a potent inhibitor of other kinases such MAPK-activated protein kinase II, protein kinase A and other kinases [Davies et al., 2000]. Moreover, recent articles describe rottlerin effects as PKC independent [Leitges et al., 2001; Susarla and Robinson, 2003; Sedmikova et al., 2006]. In this study, the effect of rottlerin on Ca^{2+} homeostasis on HMC-1^{560} cells was investigated when calcium release was induced by ionomycin or thapsigargin and his relation with the PKC.

METHODS

CHEMICALS

Thapsigargin (TG), ionomycin, [1-[r-[3-(4-methoxyphenyl)-propoxy]-1H-imidazole · HCL]] (SKF-96365), Rottlerin and GF 109203X were from Alexis Corporation (Läufelfingen, Switzerland); FURA-2 AM was from Molecular Probes (Leinden, The Netherlands); Diphenylboric acid 2-amino ethyl ester (2-ABP) and Carbonyl cyanide *p*-(trifluoromethoxy) (FCCP) were from Sigma-Aldrich (Madrid, Spain).

CELL CULTURES

HMC-1 cells were kindly provided by Dr J. Butterfield (Mayo Clinic, Rochester, MN) and maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (FBS), 100 UI/ml penicillin and 100 μg/ml streptomycin. Cells were expanded weekly.

CELL PREPARATION

For calcium measurements, cells were centrifuged (1,500 rpm, 5 min, 4°C) and then washed twice with saline solution (1,000 rpm, 5 min, 4°C). The composition of this solution was (mM): Na^+ 142.3, K^+ 5.94, Ca^{2+}1, Mg^{2+}1.2, Cl^- 126.2, HCO_3^- 22.85, HPO_4^{2-} 1.2, SO_4^{2-} 1.2, the second wash was with saline solution plus 0.1% bovine serum albumin (BSA).

In all the assays the incubation medium was equilibrated with CO_2 prior to use, to adjust the final pH to 7.20. The pH was maintained constant by burbling CO_2 during the experiment. All experiments were carried out by duplicate.

MEASUREMENTS OF CYTOSOLIC FREE CALCIUM

HMC-1 cells were loaded with FURA-2 AM (0.2 μM) in a 37°C bath, for 10 min in the saline solution plus 0.1% BSA before described. Loaded cells were washed twice with saline solution (1,500 rpm, 5 min, 4°C). Cells were allowed to attach to 22-mm glass coverslips treated with poly-L-lysine, and the coverslips were inserted into a thermostated chamber (Life Sciences Resources, UK). Cells were view using a Nikon Diphot 200 microscope equipped with epifluorescence optics (Nikon 40X-inmersion UV-Fluor objective). Addition of drugs was made by aspiration and addition of fresh bathing solution to the chamber. Cytosolic calcium concentration was obtained from the images collected by a fluorescence equipment (Life Sciences Resources). The light source was a 175 W xenon lamp, and the different wavelengths used were selected with filters. For FURA-2 AM, the excitation wavelengths were 340 and 380 nm, with emission at 505 nm. The calibration of the fluorescence values versus intracellular calcium was made according to the method of Grynkiewicz et al. [1985]. In these experiments the composition of the media used was: saline solution (mM): Na^+ 142.3, K^+ 5.94, Ca^{2+}1, Mg^{2+}1.2, Cl^- 126.2, HCO_3^- 22.85, HPO_4^{2-} 1.2, SO_4^{2-} 1.2; calcium-free saline solution, (mM): Na^+ 142.3, K^+ 5.94, Mg^{2+}1.2, Cl^- 126.2, HCO_3^- 22.85, HPO_4^{2-} 1.2, SO_4^{2-} 1.2.

STATISTICAL ANALYSIS

Results were analyzed using the Student's *t*-test for unpaired data. A probability level of 0.05 or smaller was used for statistical significance. Results were expressed as the mean ± SEM.

RESULTS

In previous studies it was observed that inhibition of PKC-σ with rottlerin (5 μM) resulted in a dramatic reduction (or even abolition) of ionomycin-induced Ca^{2+} entry in HMC-1^{560} cells, as

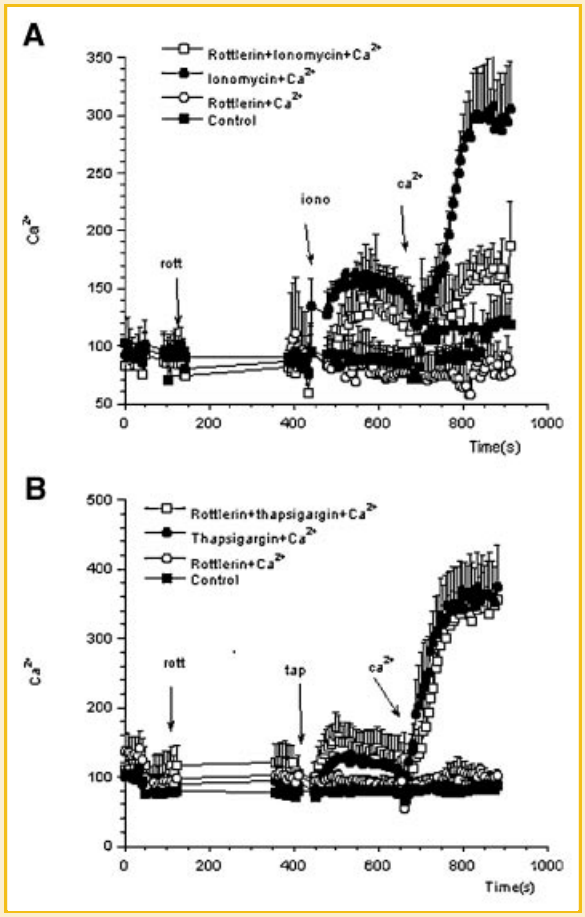

Fig. 1. Effect of rottlerin on cytosolic Ca^{2+} concentration in HMC-1^{560} cells stimulated with ionomycin (0.1 μM) or thapsigargin (2 μM). A: Cytosolic Ca^{2+} levels in cells stimulated with 0.1 μM ionomycin and incubated with 5 μM rottlerin for 5 min. First arrow indicates the addition of rottlerin. Second arrow indicates the addition of ionomycin. Third arrow indicates that Ca^{2+} is restored to the extracellular medium, after this arrow the calcium was present in the medium. B: Cytosolic Ca^{2+} profile in cells stimulated with 2 μM thapsigargin and incubated with 5 μM rottlerin. First arrow indicates the addition of rottlerin. The second arrow indicates the addition of thapsigargin. The third that Ca^{2+} is restored to the extracellular medium, after this arrow the calcium was present in the medium. Means ± SEM of three experiments (approximately 50 cells/single experiment).

Figure 1A shows [Pernas-Sueiras et al., 2006a]. In the present article it was checked if rottlerin also inhibits thapsigargin-induced Ca^{2+} entry. Thapsigargin is a tumor promoting sesquiterpene lactone that inhibits the Ca^{2}-ATPase from intracellular calcium pools, thus according to the store-operated model, producing a external Ca^{2+} influx [Thastrup et al., 1987; Hoth and Penner, 1992]. Figure 1B shows that the incubation for 5 min, with rottlerin (5 μM) does not inhibit the thapsigargin-induced Ca^{2+} entry when the ion was restored to the medium. Since initially only the dose of 5 μM rottlerin was checked the cells were incubated with higher concentrations (20 and 50 μM), but no effect in store-operated calcium influx was found (data not shown).

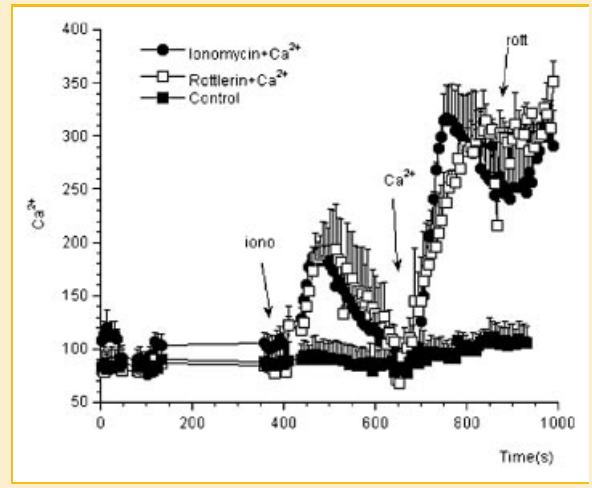

Fig. 2. Cytosolic Ca^{2+} profile in cells incubated during 5 min with 10 μM rottlerin and stimulated with 0.1 μM ionomycin. First arrow indicates the addition of ionomycin, second indicates that Ca^{2+} is restored to the medium, and third indicates the addition of rottlerin. After second arrow the calcium was present in the medium. Means ± SEM of three experiments (approximately 50 cells/single experiment).

The next step was to study if rottlerin effect on ionomycin-induced Ca^{2+} increase was due to inhibition of Ca^{2+} entry or to an increase in Ca^{2+} efflux. To answer this question rottlerin was added after Ca^{2+} was restored to the extracellular medium. Figure 2 show that rottlerin does not have any effect after Ca^{2+} was restored to the medium. It seems that rottlerin is unable to offset ionomycin-induced calcium elevation once channels are open that is, rottlerin does not affect calcium efflux once the influx is activated by ionomycin.

The relationship of ionomycin-induced intracellular Ca^{2+} store depletion with rottlerin-induced inhibition of Ca^{2+} influx was checked, and for this study the drug was added after the stores are empty. Figure 3A shows that inhibition of ionomycin-induced Ca^{2+} entry is the same as when rottlerin was added before depletion of stores. When Ca^{2+} is restored to the medium there is a high inhibition (even total ablation) of the ion influx. In the same way this relation was checked with thapsigargin-induced Ca^{2+} entry, Figure 3B, and no inhibition of calcium current was observed. The Ca^{2+} entry is still the same, about 400–500 nM. The rottlerin effect on Ca^{2+} entry does not affect the stores depletion.

SKF-96365 is a well known store-operated channel inhibitor but it also inhibits other channels over similar concentration ranges [Parekh and Putney, 2005].

Therefore the effect of both rottlerin and SKF-96365 (30 μM) were compared on ionomycin induced Ca^{2+} influx. Figure 4 show that the incubation for 5 min with SKF-96365 did not show the same effect as rottlerin. There is a Ca^{2+} influx reduction when the ion was restored to the medium but the inhibition is not as evident as for rottlerin (a nearly complete abolition), since a small Ca^{2+} entry (over 250–200 nM) in the presence of SKF-96365 is observed. While in previous articles it is shown that SKF-96365 has the same effect over thapsigargin and ionomycin induced Ca^{2+} signal [Pernas-Sueiras et al., 2006b].

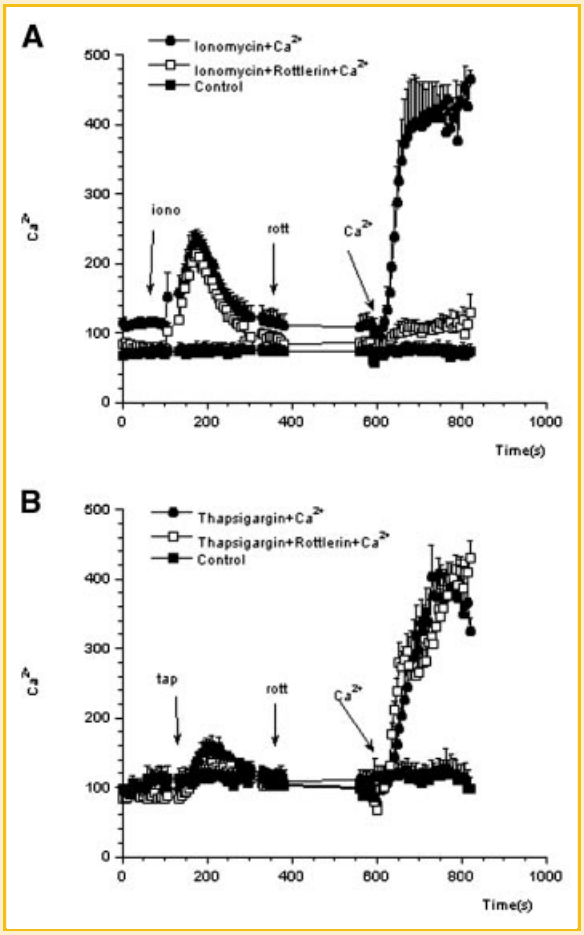

Fig. 3. Effect of rottlerin on cytosolic Ca^{2+} concentration in HMC-1^{560} cells stimulated with ionomycin (0.1 μM) or thapsigargin (2 μM). A: Cytosolic Ca^{2+} levels in cells stimulated with 0.1 μM ionomycin and incubated with 10 μM rottlerin during 5 min: First arrow indicates the addition of ionomycin. Second indicates the addition of rottlerin, and third indicates that Ca^{2+} is restored to the medium, after this arrow the calcium was present in the medium. B: Cytosolic Ca^{2+} levels in cells stimulated with 2 μM thapsigargin and incubated with 10 μM rottlerin during 5 min. First arrow indicates the addition of thapsigargin. Second indicates the addition of rottlerin, and third indicates that Ca^{2+} is restored to the medium, after this arrow the calcium was present in the medium. Means ± SEM of three experiments (approximately 50 cells/single experiment).

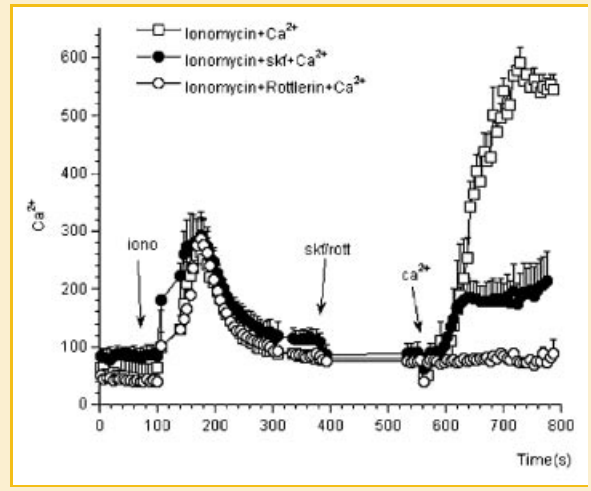

Fig. 4. Cytosolic Ca^{2+} profile in cells incubated with 10 μM rottlerin or 30 μM SKF-96365 during 5 min and stimulated with 0.1 μM ionomycin. First arrow indicates the addition of ionomycin, second indicates the addition of rottlerin or SKF-96365, and third indicates that Ca^{2+} is restored to the medium, after this arrow the calcium was present in the medium. Means ± SEM of three experiments (approximately 50 cells/single experiment).

It has been recently reported a different effect of rottlerin independent of PKC-δ [Leitges et al., 2001; Susarla and Robinson, 2003; Sedmikova et al., 2006] and there are serious doubts about rottlerin PKC-δ inhibition. To check if the rottlerin effect in Ca^{2+} entry is dependent or independent of PKC-δ the cells were incubated with GF 109203X, a well know non-specific PKC inhibitor. GF 109203X, 500 nM, inhibits Ca^{2+} dependent and independent PKC isoforms (PKC-δ included). Figure 5 shows that the incubation of cells for 5 min with GF 109203X (500 nM) does not affect the Ca^{2+} profile when cells were stimulated either with ionomycin (Fig. 5A), or thapsigargin (Fig. 5B).

Finally, rottlerin can induce PKC-δ independent effects. In this sense it was observed a direct effect of rottlerin in uncoupling mitochondrial respiration from oxidative phosphorylation (uncouples mitochondrial ATP synthesis) [Soltoff, 2001]. Their mitochondrial effect was compared with carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). FCCP is a protonophore (H^+ ionophore) and a known uncoupler of oxidative phosphorylation in mitochondria. This drug induced a depolarization of the plasma and mitochondrial membranes. Therefore it was checked if FCCP has some effect in ionomycin or thapsigargin-induced Ca^{2+} entry and if this is similar to the results obtained with rottlerin. For this the cells were incubated for 5 min, after intracellular pools were depleted by ionomycin or thapsigargin, with FCCP (5 μM). Figure 6A shows that FCCP does not have effect in thapsigargin-induced Ca^{2+} influx. However as Figure 6B shows after ionomycin addition, a strong inhibition of the ion entry is observed when this is restored to the medium, in the same way as observed for rottlerin when the Ca^{2+} entry is induced by ionomycin (Fig. 1A).

DISCUSSION

In this work the rottlerin effects in CRAC currents produced by ionomycin and thapsigargin were studied. In addition it was studied the relationship between this effect and the possible regulatory role of PKC-δ.

In previous works it was shown a very remarkable finding, that inhibition of PKC-δ with rottlerin resulted in a reduction of ionomycin-induced Ca^{2+} entry [Pernas-Sueiras et al., 2006a]. But a surprising fact is that rottlerin failed in showing any effect in thapsigargin-induced Ca^{2+} influx. Ionomycin (Ca^{2+} ionophore) and thapsigargin (SERCA pump blocker) induced a rise in intracellular Ca^{2+} concentration by store depletion, and this rise activated SOC channels (Ca^{2+}-activated cation channels

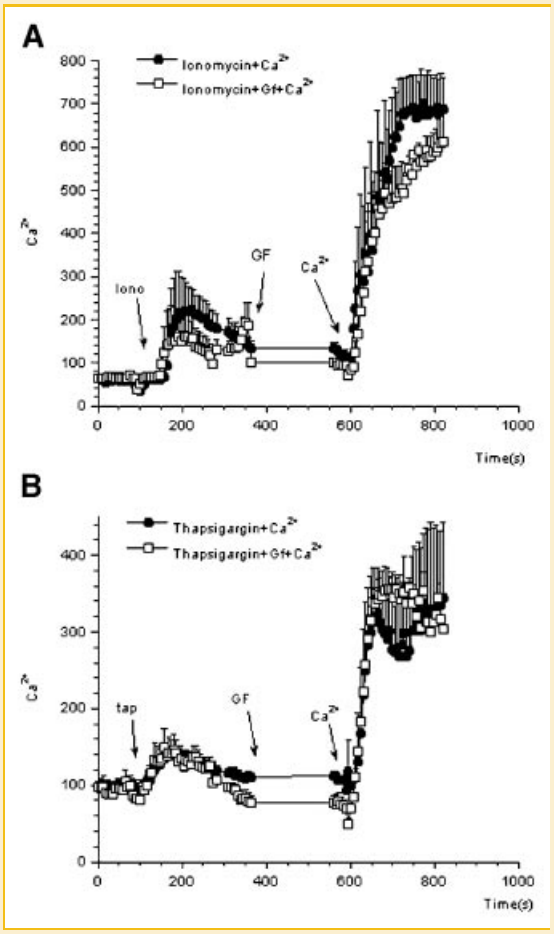

Fig. 5. Effect of GF 109203X on cytosolic Ca^{2+} concentration in HMC-1^{560} stimulated with ionomycin or thapsigargin. A: Cytosolic Ca^{2+} levels in cells stimulated with 0.1 μM ionomycin and incubated with 500 nM GF 109203X during 5 min. First arrow indicates the addition of ionomycin, the second the addition of GF 109203X, and the third that Ca^{2+} is restored to the extracellular medium, after this arrow the calcium was present in the medium. B: Cytosolic Ca^{2+} profile in cells stimulated with 2 μM thapsigargin and incubated with 500 nM GF 109203X. First arrow indicates the addition of thapsigargin. The second the addition of GF 109203X, and the third that Ca^{2+} is restored to the extracellular medium, after this arrow the calcium was present in the medium. Means ± SEM of three experiments (approximately 50 cells/single experiment).

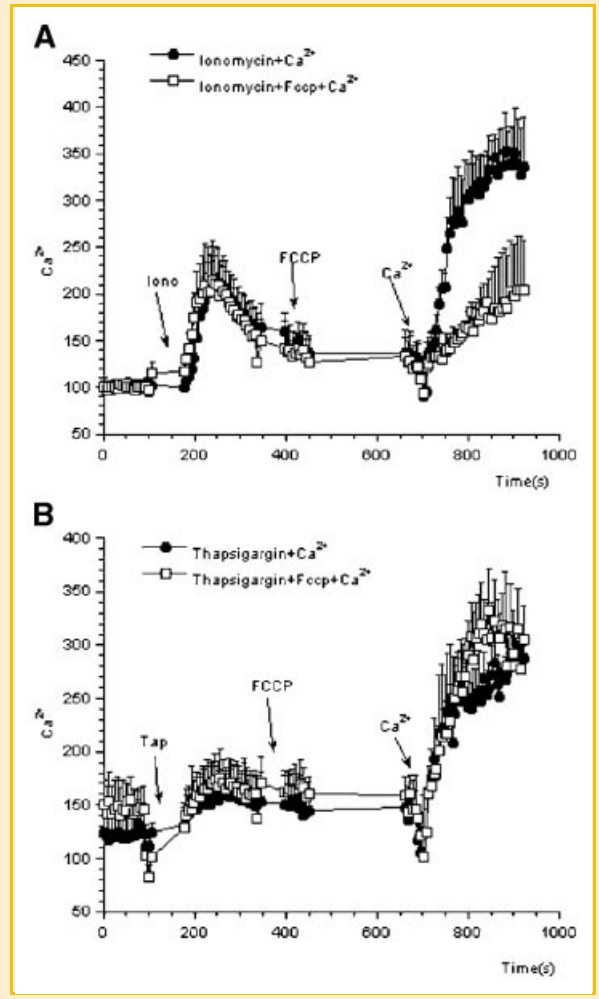

Fig. 6. Effect of FCCP on cytosolic Ca^{2+} concentration in HMC-1^{560} stimulated with ionomycin or thapsigargin. A: Cytosolic Ca^{2+} levels in cells stimulated with 2 μM thapsigargin and incubated with 5 μM FCCP during 5 min. First arrow indicates the addition of thapsigargin. The second the addition of FCCP, and the third that Ca^{2+} is restored to the extracellular medium, after this arrow the calcium was present in the medium. B: Cytosolic Ca^{2+} profile in cells stimulated with 0.1 μM ionomycin and incubated with 5 μM FCCP during 5 min. First arrow indicates the addition of ionomycin. The second the addition of FCCP, and the third that Ca^{2+} is restored to the extracellular medium, after this arrow the calcium was present in the medium. Means ± SEM of three experiments (approximately 50 cells/single experiment).

permeable to Ca^{2+}). Ionomycin depleted the stores by do permeable the endoplasmic membrane whereas thapsigargin inhibits the endoplasmic Ca^{2+}-ATPase preventing stores refilling. Effect of these compounds was performed with successive addition of thapsigargin and ionomycin, that interact with thapsigargin sensitive and insensitive intracellular Ca^{2+} stores [Pernas-Sueiras et al., 2005], in good agreement with findings in other cellular models [Soboloff and Berger, 2002; Turner et al., 2003]. The fact that rottlerin has effect on ionomycin and not on thapsigargin-induced Ca^{2+} entry could be explained in three ways, because they operate in different stores, because the store depletion activates different channels, or if the activation route is not the same. Actually it is not still exactly known how many types of CRAC channels are. It is known that there are at least three CRACM homologous and it has been reported that all three CRACM homologous exhibit distinct properties in terms of selectivity for Ca^{2+} and Na^{+}, different pharmacological effects in response to inhibitors (like 2-APB), and strikingly different feedback regulation by intracellular Ca^{2+} [Lis et al., 2007].

Another issue to know is if rottlerin effect is mediated in some way by store depletion. Rottlerin shows the same effect when the incubation was done after and before store depletion. This means that the inhibitory effect of rottlerin is directly related with SOC channels or the SOC channel activation, without affecting stores depletion. Moreover rottlerin had no effect in thapsigargin-induced Ca^{2+} entry when the incubation was performed after store

depletion. This fact suggests the possibility that the differences between ionomycin and thapsigargin lay in the way SOC channels are activated and not the thapsigargin or ionomycin sensitive stores. Also our data show that when rottlerin addition was done after Ca^{2+} influx is already beginning no inhibition is reported. That is rottlerin is unable to offset ionomycin-induced elevation in calcium once channels are open, that is, rottlerin is not able to show the Ca^{2+} effect when the SOC channel activated by ionomycin had been open.

In order to compare data obtained with rottlerin, we carried out the same experiments using the SOC channels inhibitor SKF-96365. SKF-96365 is used for SOC studies but it is also known that other Ca^{2+} channels can be blocked and therefore results about SOC channels should be taken with caution [Parekh and Putney, 2005].

SKF and rottlerin both inhibit the ionomycin-induced Ca^{2+} entry, but on the contrary rottlerin does not modify the effect of thapsigargin. Hence, SKF-96365 and rottlerin seem to have different targets.

On the other hand, it has been reported that rottlerin PKC-δ inhibition (LC Laboratories report), could be attributed to an impurity present in PKC-δ extract. GF 109203X, a well known non-specific PKC inhibitor, did not show any effect in ionomycin or thapsigargin-induced Ca^{2+}. GF 109203X inhibits dependent and independent Ca^{2+} PKC isoforms including also the δ isoform at the concentration used in our experiments. The absence of effect suggest that the rottlerin effect that we observed should be PKC-δ independent. It is a remarkable fact that GF 109203X did not show any effect in the Ca^{2+} profile of HMC-1^{560} when previous articles report that the phorbol ester 12-myristate13-acetate (PMA) enhances ionomycin and thapsigargin-induced Ca^{2+} entry. This is not a direct action in Ca^{2+} levels, since PMA alone did not modified the Ca^{2+} profile [Pernas-Sueiras et al., 2006b]. Protein Kinase C has different effects in Ca^{2+} signaling, it has bee described that PMA inhibits Ca^{2+} entry [Baranska et al., 1995; Shibata et al., 1996; Haverstick et al., 1997], others describe a dual regulation or even no effect in Ca^{2+} profile [Petersen and Berridge, 1994; Wolfe et al., 1996; Scott et al., 1998]. Therefore the role of PKC in Ca^{2+} activation is still unresolved.

The results indicate that rottlerin effect on ionomycin is PKC-δ independent, in recent years some works had been written about rottlerin PKC-δ independent roles. It was reported that rottlerin inhibits the astrocyte glutamate transport activity by a possible PKC independent effect [Susarla and Robinson, 2003]. In addition rottlerin did show the same effects in wild cells and PKC-δ negative cells, indicating that presence of other target kinases beyond the PKC family [Leitges et al., 2001]. In addition to this, rottlerin has been reported as a mitochondrial uncoupler that increases the rate of oxygen consumption, in a similar way as ADP or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), to initiate oxidative phosphorylation [Kayali et al., 2002; Liao et al., 2005; Tapia et al., 2006; Kurosu et al., 2007]. Rottlerin and FCCP mimicked the same effects in isolated rat liver mitochondria [Soltoff, 2001].

In the present article FCCP inhibited the ionomycin-induced Ca^{2+} entry and failed to show any effect on thapsigargin-induced Ca^{2+} entry, in a similar way than rottlerin. FCCP is a protonophore that induces depolarization of plasma and mitochondrial membranes, our data suggest the mitochondria as a target for rottlerin effect on ionomycin-induced Ca^{2+} entry. Changes in mitochondrial Ca^{2+} levels and dynamics are very important for cell physiology. Respiring mitochondria is an important modulator in activation and inactivation of CRAC channels, since it regulates intracellular Ca^{2+} buffering, that plays a pivotal role in CRAC channels state and it can affect ICRAC [Gilabert and Parekh, 2000; Glitsch et al., 2002].

In summary rottlerin has a calcium effect in HMC-1^{560} that is linked to ionomycin-induced Ca^{2+} entry mechanism, but it does not affect the thapsigargin-induced ion entry. This effect is PKC-δ independent and without relation with the store depletion. The results point to the respiring mitochondria as the rottlerin target.

Further work is needed to establish and identify the functional role of mitochondria in ionomycin-induced I CRAC and the difference between ionomycin and thapsigargin SOC channel activation in HMC-1^{560}.

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