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Fcε receptor mediated Ca^{2+} influx into mast cells is modulated by the concentration of cytosolic free Ca^{2+} ions

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The relationship between the Fcε receptor mediated stimulation of mast cells and the Ca^{2+} signal it induces were studied using thapsigargin (TG), a blocker of the endoplasmic reticulum Ca^{2+} pump. TG induced, in mucosal mast cells (RBL-2H3 line), a dose-dependent and an $InsP_3$-independent increase in $[Ca^{2+}]_i$ (from resting levels of 83–150 nM to 600–680 nM), and a secretory response amounting to 30–50% of that observed upon FcεRI clustering. The TG induced rise of $[Ca^{2+}]_i$ is most probably provided by both arrest of its uptake by the endoplasmic reticulum and influx from the medium. Thus, Ca^{2+} influx in mast cells may be modulated by the $[Ca^{2+}]_i$ level.

Mast cell; Rat basophilic leukemia cell (RBL-2H3); Type 1 Fcε receptor; Ca^{2+} influx; Signal transduction; Thapsigargin

1. INTRODUCTION

A transient increase in cytosolic free Ca^{2+} ion concentrations, $[Ca^{2+}]_i$, is a common stimulus to response coupling element observed in a variety of cells [1–3]. Generally, it is characterized by two phases: an initial rapid spike followed by a sustained, elevated phase. The initial spike has been found to be mainly a result of Ca^{2+} release from intracellular stores mediated by inositol 1,4,5-trisphosphate ($InsP_3$). This in turn is generated by receptor mediated activation of phospholipase Cγ1 [4–6]. The second phase of elevated $[Ca^{2+}]_i$ is a plateau caused mainly by Ca^{2+} influx from the extracellular medium, probably through the opening of plasma membrane channels [7–9]. Little is known about the biochemical events which initiate the second phase and a possible linkage between the two phases of the Ca^{2+} response. However, involvement of different processes, including those resulting from mobilization of intracellular Ca^{2+} pools have been postulated [4,10–14].

The rat mucosal mast cell line RBL-2H3 provides a useful model for the study of immunological stimulus–response coupling process and in particular of the receptor-mediated Ca^{2+} signal. Clustering of the type 1 receptor for IgE (FcεRI) has also been shown to induce a biphasic $[Ca^{2+}]_i$ response as described above [15], and culminates in the secretion of mediators of immediate hypersensitivity [16]. This secretory process has an absolute requirement for Ca^{2+} ions in the extracellular medium [16,17]. Early studies have shown that raising the $[Ca^{2+}]_i$ by ion specific ionophores is an effective means for circumventing the above biochemical pathway and leading to a similar secretory response [18,19]. The action of Ca^{2+} ionophores clearly demonstrated the essential role played by free cytosolic levels of Ca^{2+} ions. Yet, the ionophores compromise the physiological compartmentation of the cell and hence, could not help in resolving the specific pools and pathways involved in the FCεRI mediated process.

To study the consequences of intracellular Ca^{2+} stores depletion, especially the relations between Ca^{2+} mobilization from intra- and extracellular sources, we employed thapsigargin (TG), a sesquiterpene lacton which has been shown to increase $[Ca^{2+}]_i$, apparently, by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase [20]. In previous studies TG has been found to increase $[Ca^{2+}]_i$ in a broad spectrum of unrelated cell types; neutrophils [21], blood platelets [22], NG-115-401L neuronal cells [23], parotid acinar cells [24] and T lymphocytes [25]. Its action was shown to be, at least in the latter three cell types, independent of phosphatidylinositides hydrolysis. In parotid acinar cells and T lymphocytes, TG was also shown to induce the sustained elevated $[Ca^{2+}]_i$ signal [24,25]. More relevant to our experimental model, mast cells derived from rat peritoneum, mesentery, lung and heart were all found to secrete serotonin in response to TG application [26]. However, the underlying process has not been further examined. Here we report studies where TG was em-

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Abbreviations: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} ion concentration; EGTA, ethylene glycol diamine *N,N,N'N'*-tetraacetic acid; FcεRI, Fcε-receptor, type 1; FCS, fetal calf serum; $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_4$, inositol 1,3,4,5-tetrakisphosphate; RBL-2H3, rat basophilic leukemia cells, subline 2H3; TG, thapsigargin.

ployed to examine the relationship between the FcεRI stimulus and the Ca^{2+} signal it induces.

The results demonstrate that TG induced mobilization of Ca^{2+} from intracellular stores that can quantitatively account for the elevation of $[Ca^{2+}]_i$ and for the influx of these ions in mucosal mast cells of the RBL-2H3 line. However, the TG induced rise in $[Ca^{2+}]_i$ does not yield as high a secretory response as FcεRI clustering. Hence, the involvement of further second messengers is apparently required. Furthermore, the results indicate that depletion of intracellular Ca^{2+} pools may play a major role in the opening of cell membrane Ca^{2+} channels.

2. MATERIALS AND METHODS

2.1. *Materials*

Powdered culture media were purchased from Gibco, Grand Island, NY, USA. Fetal calf serum, glutamine and penicillin-streptomycin mixture were obtained from Bio-Lab, Jerusalem, Israel. Triton X-100 and *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine were from Sigma Chemical Co., St. Louis, MO, and EGTA and DMSO from Merck, Darmstadt, Germany. The Ca^{2+} indicators, fluo-3-acetoxy-methyl ester (fluo-3/AM) and Quin-2-acetoxy-methyl ester (Quin-2/AM), were obtained from Molecular Probes, OR. DNP_{11}-BSA, was prepared in our laboratory by derivatization of bovine serum albumin (Sigma) with 2,4-dinitro-benzene-sulfonic acid (Merck). Murine DNP-specific IgE class mAb A_2 [27], was grown as ascites in mice. Thapsigargin (TG), was from Calbiochem Biochemicals, San Diego, CA. $^{45}CaCl_2$ and [^{3}H]myo-inositol were purchased from Amersham, UK.

2.2. *Cells*

Rat mucosal mast cells, subline RBL-2H3 [28], were maintained in Eagle's minimal essential medium (MEM) with Earle's salts supplemented by 10% FCS, 2 mM glutamine and antibiotics in a humidified atmosphere with 7% CO_2 at 37°C. During the experiments, cells were maintained in Tyrode's buffer: 137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 5.6 mM glucose, 10 mM HEPES, 0.1% BSA, pH 7.4. Adherent cells were harvested following 15 min incubation with 10 mM EDTA in MEM. Cell viability was assessed by the exclusion of Trypan blue dye (final concentration of 0.05%) and was invariably greater than 96%.

2.3. *Secretory response of RBL-2H3 cells*

The secretion of mediators by RBL-2H3 cells in response to stimulation with antigen (DNP_{11}-BSA) or with TG was monitored by measuring the activity of the granular enzyme β-hexosaminidase. Murine DNP-specific mAb IgE, A_2, was added to cells (3 μl of ascites/10^6 cells) in 96-well plates (10^5 cells in 100 μl MEM/well). On the following day, monolayers were washed three times with Tyrode's buffer and subjected to the above mentioned stimuli at 37°C. 20 μl supernatant aliquots were taken to a separate plate and 50 μl of substrate solution (1.3 mg/ml *p*-nitrophenyl-*N*-acetyl-β-D-glucosamine in 0.1 M sodium citrate, pH 4.5) were added. The plates were then incubated for 60 min at 37°C and the reaction terminated by addition of 150 μl of 0.2 M glycine, pH 10.7. The colour formed by substrate hydrolysis was measured at 405 nm in an ELISA reader.

2.4. *$[Ca^{2+}]_i$ determination using fluo-3/AM*

RBL-2H3 cells from confluent cultures (preincubated for 3 h with the DNP-specific IgE, 3 μl/10^6 cells) were washed, suspended in Tyrode's buffer at 10^7 cells/ml and incubated with 5 μM fluo-3-acetoxymethyl ester (1 mM stock solution in DMSO) for 30 min at 37°C, with gentle shaking. After washing with the same buffer, 1 ml aliquots of 10^6 cells/ml were transferred to a quartz cuvette (7 × 7 mm) and the fluorescence intensity (F_{exp}) was monitored in a Perkin-Elmer MPF-44A spectrofluorometer (excitation at 490 nm, emission at 530 nm). The cuvette was thermostatted at 37°C and an electrically driven mini-stirrer inserted into the cuvette ensured continuous homogeneity of the suspension. In the beginning and at the end of each experiment, fluorescence of 1 ml cell suspension's supernatant was measured after 10 μl of 15 mM $MnCl_2$ were added to quench fluo-3 fluorescence. These two values were used for correcting, by interpolation, for extracellular fluo-3 ($F_{out,Mn}$). After each experiment, the cells were lysed in 1% Triton X-100 to saturate the fluo-3 with Ca^{2+} and subjected to 2 mM $MnCl_2$ ($F_{final,Mn}$). A fluo-3-free cell's sample was used to determine the background fluorescence (F_{bkg}).

$[Ca^{2+}]_i$ was calculated according to the following expression [29]:

$$[Ca^{2+}] = K_d(F_{in}-F_{min})/(F_{max}-F_{in})$$

K_d, the dissociation constant for Ca^{2+}-bound fluo-3, is 400 nM [30]. The Mn^{2+}/fluo-3 complex is 8 times as fluorescent as the metal-free dye, but only one-fifth as intense as the Ca^{2+}/fluo-3 complex [30,31]. Therefore, F_{max}, F_{min} and F_{in} can be calculated as follows:

$$F_{max} = 5(F_{final,Mn}-F_{out,Mn}-F_{bgk})$$

$$F_{min} = F_{max}/40$$

$$F_{in} = F_{exp}-F_{out,Mn}-F_{bkg}$$

2.5. *$^{45}Ca^{2+}$-uptake measurements*

$^{45}Ca^{2+}$ uptake by monolayers of RBL-2H3 cells grown in 16-mm diameter wells was measured essentially as described [32]. To provide intracellular Ca^{2+} buffering, the cells were first loaded with 100 μM of the chelating agent, Quin-2/AM (10 mM stock solution in DMSO), by preincubation for 30 min at 37°C in Tyrode's buffer. Following this, the cells' monolayers were washed three times with the same buffer and stimulated with specific antigen (DNP_{11}-BSA) or with TG, at the indicated concentrations, in Tyrode supplemented with 50 μCi/ml (10^6–10^7 cpm) $^{45}Ca^{2+}$ at 37°C. After the indicated time, supernatants were aspirated, monolayers were washed rapidly with 3 × 2 ml portions of ice-cold quenching buffer (135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, pH 7.4) and lysed in 0.5 ml of 0.1% Triton X-100. Then the β-radiation of 0.4 ml lysate samples was determined and the measured cpm converted into nmol Ca^{2+}/10^6 cells.

2.6. *Inositol phosphates (InsP) production*

Measurements of InsP production were performed as previously described [6]. Briefly, RBL cells incubated overnight in medium 199 containing 10% dialysed FCS, [^{3}H]myo-inositol (3 μCi/ml, 10^6 cell/ml) and IgE (3 μl ascites/10^6 cells), were harvested, washed and incubated for 15 min at 37°C in Tyrode's buffer containing 5 mM LiCl. 2 × 10^6 cells/350 μl aliquots were stimulated with either DNP-BSA or TG at 37°C for the indicated times. Reaction was stopped by addition of 1.5 ml ice-cold chloroform–methanol (1:2) acidified with 0.1 N HCl. After addition of further 0.5 ml of chloroform and 0.5 ml of water, 1.5 ml of the upper layer were applied to pre-equilibrated (extensive washing with 2 M ammonium formate, followed by washing with water) Dowex 1-X8 anion-exchange columns (3 ml) for separating the inositol phosphates. Columns were washed with 10 ml of water and the inositol monophosphate ($InsP_1$), biphosphate ($InsP_2$) and trisphosphate ($InsP_3$) were sequentially eluted with 10 ml of 0.2 M ammonium formate, 0.1 N formic acid ($InsP_1$); 5 ml of 0.4 M ammonium formate, 0.1 N formic acid ($InsP_2$); and 5 ml of 1 M ammonium formate, 0.1 N formic acid ($InsP_3$). The eluates were collected in vials for liquid scintillation counting.

3. RESULTS

TG was found to cause a non-cytolitic (cell viability >96% even at 5 μM TG), dose-dependent secretory re-

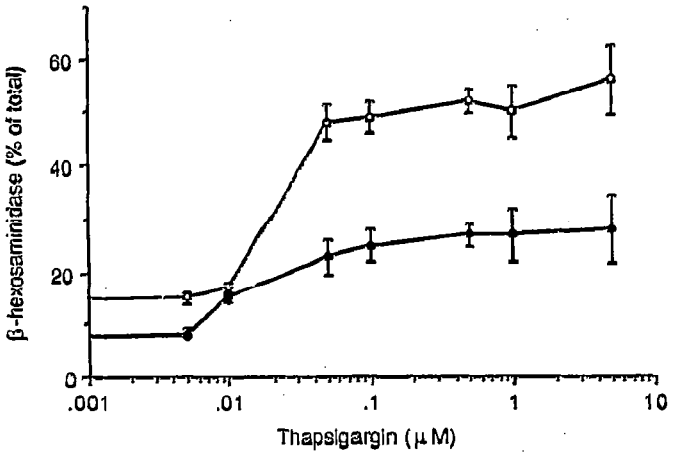

Fig. 1. Dose dependence of TG induced secretory response of RBL-2H3 cells. Cell monolayers incubated overnight with the DNP-specific monoclonal A_2IgE were treated for 30 min at 37°C with the indicated TG concentrations in the absence (•) or presence (○) of 2 ng/ml DNP_{11}-BSA. Secretion was assayed by β-hexosaminidase activity appearing in the supernatants as detailed under Materials and Methods. The data presented are the mean of 4 replicates ± S.D. and were obtained in a typical experiment out of a series of three independent ones.

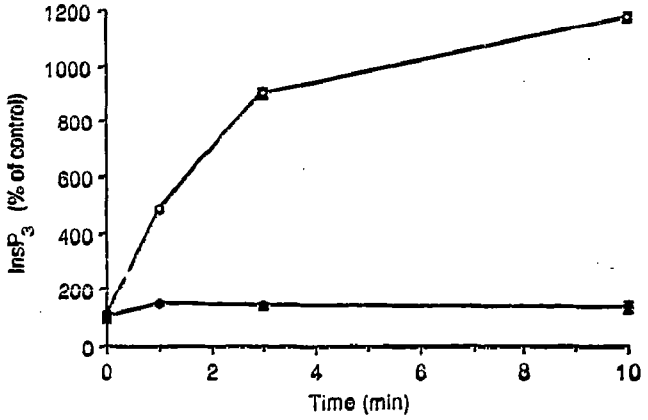

Fig. 3. Rate of IP_3 formation induced either by FcεRI clustering or by TG. RBL cells (5 × 10^6/ml), preincubated overnight with 4 μCi/ml [^{3}H]inositol and DNP-specific monoclonal A_2IgE, followed by 15 min incubation with 5 mM LiCl, were stimulated for the indicated length of time with either (○) 10 ng/ml DNP_{11}-BSA or (•) 100 nM TG. Reaction was stopped by ice-cold chloroform–methanol and inositol phosphates were extracted and separated by anion-exchange chromatography as detailed under Materials and Methods. Results are expressed as the content of $InsP_3$ relative to its level in samples of unstimulated cells. Data are the mean of triplicates ± S.D. and were obtained in a typical experiment out of two independent ones.

sponse of mucosal mast cells of the RBL-2H3 line. Maximal β-hexosaminidase secretion (net 18–30% of cells' total content) was observed already at TG concentration of 100 nM (Fig. 1). The level of TG induced mediator release ranged from 30% to 50% (n = 7) of that observed upon FcεRI clustering and showed absolute requirement for the presence of Ca^{2+} ions in the extracellular medium (Fig. 2). No significant increase in the levels of $InsP_3$ could be resolved in the presence of TG doses effective in inducing secretion (130 ± 16% of control compared with 1200 ± 16% induced upon antigenic stimulation; Fig. 3). Combined stimulation by both FcεRI clustering (by DNP-BSA added to cells preincubated with specific IgE) and TG, yielded synergism in secretion (Figs. 2 and 4). In addition, TG (100 nM) increased the rate of secretion induced by suboptimal doses of antigen (1 ng/ml). Thus, maximal secretion was reached already 15 min after TG addition to cells treated with 2 ng/ml DNP-BSA at the beginning of the experiments, whereas response to antigen alone peaks at 30 ± 5 min. Addition of similar doses of DNP-BSA to TG pretreated cells had, however, a smaller effect on the response rate (Fig. 4).

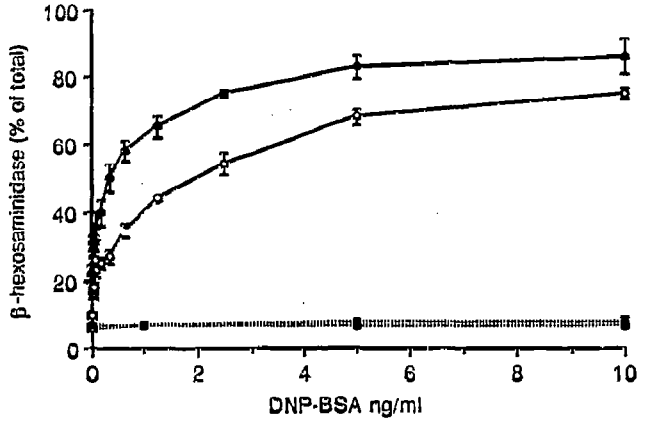

Fig. 2. Synergistic secretory response to the combined stimulation of TG and FCεRI-clustering. RBL cell monolayers incubated overnight with DNP-specific monoclonal A_2IgE were stimulated by DNP_{11}-BSA at the indicated concentrations in the absence (○) or presence (•) of 100 nM TG; with (———) or without (····) 2 mM $CaCl_2$ in the medium. Secretion was assayed by β-hexosaminidase activity in the supernatants as detailed under Materials and Methods. Points are the mean of four replicates ± S.D. and were obtained in a typical experiment out of a series of four independent ones.

Cytosolic free Ca^{2+} ion concentrations, $[Ca^{2+}]_i$, were measured fluorometrically in Fluo 3/AM loaded RBL-2H3 cells. $[Ca^{2+}]_i$ increased immediately following the addition of 100 nM TG, from the basal levels (83–150 nM) of resting cells to 600–680 nM (n = 4; Fig. 5). The maximal levels of $[Ca^{2+}]_i$ were reached at about 120 s after TG addition with half-maximal response observed at 60 s. The maximal levels observed have declined gradually (within 6 min) to a prolonged elevated plateau of $[Ca^{2+}]_i$ = 350 nM which was maintained for at least 15 more min. This pattern of $[Ca^{2+}]_i$ signal was distinctly different from that observed in response to FcεRI clustering (Fig. 5), where a similar initial increase in $[Ca^{2+}]_i$ levels is reached almost as fast (30–60 s), but it also declines faster (after ca. 1 min) to a lower sustained plateau (250–330 nM) which is maintained for at least 10 min (Fig. 5a).

TG (100 nM) added to RBL-2H3 cells 2 min after

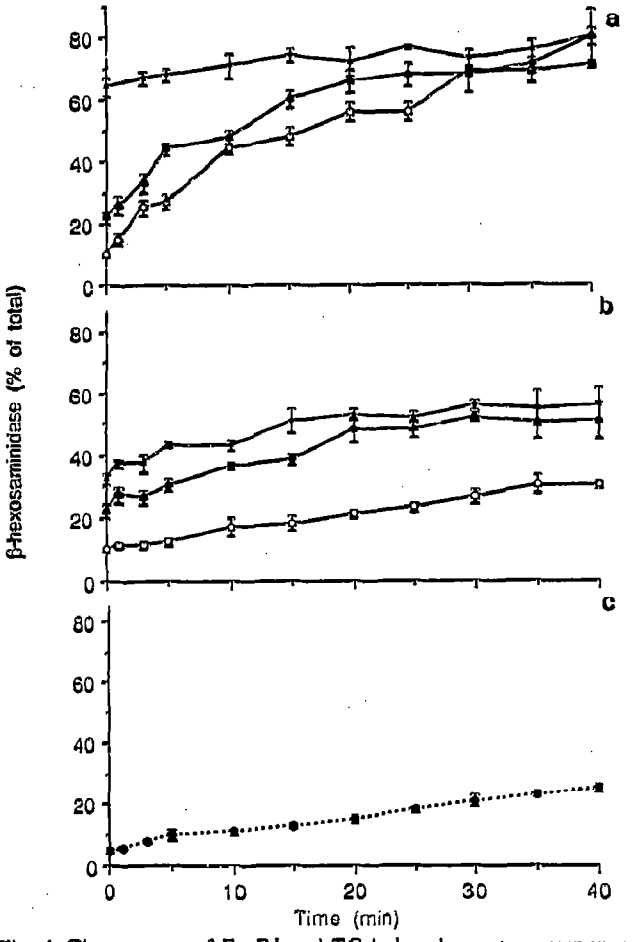

Fig. 4. Time course of FcεRI and TG-induced secretory response of RBL cells. Cell monolayers preincubated with DNP-specific monoclonal A_2IgE were stimulated for the indicated length of time by DNP_{11}-BSA with (●) or without (○) TG 100 nM presence. Alternatively, (◇) TG was added for the indicated time to cells triggered with DNP_{11}-BSA at time zero. DNP_{11}-BSA concentration: (a) 10 ng/ml, (b) 1 ng/ml DNP_{11}-BSA; (c) cells incubated with (--●--) 100 nM TG for the indicated time. The cells' secretion was assayed by β-hexosaminidase activity appearing in the supernatant as detailed under Materials and Methods. Data are the mean of four replicates ± S.D. and were obtained in a typical experiment out of a series of four independent ones.

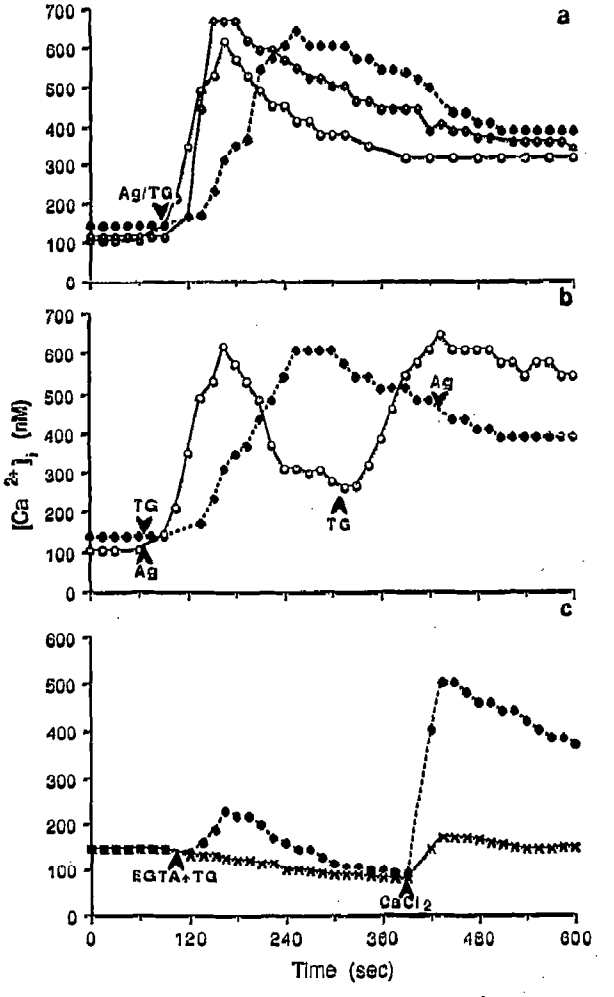

Fig. 5. FcεRI clustering and TG-mediated rise of $[Ca^{2+}]_i$ in RBL cells. Cells (10^7/ml) indicated for 3–18 h with the DNP-specific monoclonal A_2IgE were loaded with the dye fluo-3/AM (5 μM) by further incubation for 30 min at 37°C. After washing off the residual external indicator, 1 ml aliquots of cell suspension (10^6/ml) were transferred to a thermostatted cuvette and were treated at the indicated time (arrow) with: (a) 10 ng/ml DNP_{11}-BSA (○), 100 nM TG (●) or simultaneous addition of both the above reagents (◇). (b) 100 nM TG added during the second phase of response to 10 ng/ml DNP_{11}-BSA stimulation (○) or 10 ng/ml DNP_{11}-BSA added after the maximal response to 100 nM TG (●); (c) 3 mM EGTA added to the cells' suspension with (●) or without (×) 100 nM TG stimulation. In both cases 2 mM $CaCl_2$ were added after 4 min. The fluorescence was monitored using spectrofluorometer (excitation 490 nm, emission 530 nm). $[Ca^{2+}]_i$ was calculated as described under Materials and Methods. Shown is a typical experiment out of a series of five (a,b) or two (c) independent ones.

stimulation with antigen, i.e. during the elevated $[Ca^{2+}]_i$ plateau period caused by the FcεRI clustering, induced a further increase in $[Ca^{2+}]_i$ from about 250 nM up to 650 nM (Fig. 5b). In contrast, when an FcεRI stimulus (10 ng/ml DNP-BSA) was applied to cells after TG, practically no further changes in $[Ca^{2+}]_i$ were observed (Fig. 5b). Simultaneous stimulation with optimal doses of, both TG (100 nM) and DNP-BSA (10 ng/ml) resulted in a rapid elevation of $[Ca^{2+}]_i$ similar to that seen with antigen alone, but the following plateau was higher and more prolonged, reminiscent of that observed with TG (Fig. 5a).

Upon lowering the extracellular Ca^{2+} ions concentration by adding 3 mM EGTA to the medium prior to TG stimulation, a markedly lower (250–300 nM) and shorter rise in $[Ca^{2+}]_i$ was observed, which returned to basal levels within 120 s (Fig. 5c). These findings underscore the capacity of TG to mobilize intracellular Ca^{2+}. Re-administration of Ca^{2+} (2 mM $CaCl_2$) to the cells' medium induced an instantaneous and sustained increase in $[Ca^{2+}]_i$, dramatically higher than that observed when 2 mM $CaCl_2$ is added to the medium of non-

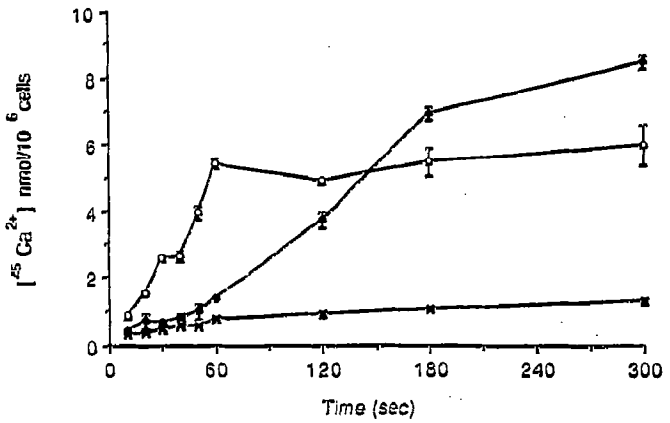

Fig. 6. FcεRI- and TG-mediated $^{45}Ca^{2+}$ uptake. Cell monolayers (10^6/ml) carrying the DNP-specific monoclonal A_2IgE were incubated at 37°C with 100 μM Quin-2/AM for 30 min, washed and either left as untreated controls (×) or stimulated with 10 ng/ml DNP_{11}-BSA (○) or 100 nM TG (◆) in Tyrode's buffer containing 50 μCi/ml $^{45}Ca^{2+}$. After the indicated time at 37°C, reaction was terminated as described under Materials and Methods. The β-radiation of 0.4 ml cell-lysate sample was determined and the measured cpm converted into nmol $Ca^{2+}/10^6$ cells. Results of a representative experiment (one of three independent ones) are presented as the mean of triplicates ± S.D.

stimulated cells pretreated with 3 mM EGTA (510 nM and 170 nM, respectively).

Ca^{2+} influx was studied by measuring $^{45}Ca^{2+}$ uptake by RBL-2H3 cells which were preloaded with 100 μM of the Ca^{2+} chelating Quin-2/AM to provide intracellular Ca^{2+} buffering [32]. As shown in Fig. 6, stimulation of the cells by FcεRI clustering (with IgE and 10 ng/ml DNP-BSA) resulted in an immediate $^{45}Ca^{2+}$ uptake that reached its maximal value at ca. 60 s after antigen addition. It proceeded at a rate of 0.09 nmol/10^6 cells · s. Stimulation with 1 μM TG induced a slower uptake (0.06 nmol/10^6 cells · s) that approached maximal levels 180 s after stimulation. $^{45}Ca^{2+}$ uptake by these TG treated cells continued at a slow rate (0.01 nmol/10^6 cells · s) without reaching plateau throughout the experiment (30 min).

4. DISCUSSION

Resolution of the mechanism controlling Ca^{2+} influx across the plasma membrane of rat mucosal mast cells (RBL-2H3) is a main objective of our studies. This process is essential for the FcεRI-mediated rise in $[Ca^{2+}]_i$ and the ensuing secretory response [9,15,16,32]. Recent investigations, in other cell types, have shown the existence of at least two, apparently distinct, mechanisms of Ca^{2+} influx control. One depends on the level of $[Ca^{2+}]$ and/or the saturation state of the intracellular Ca^{2+} stores [11,13], while the second is directly coupled to receptor activation, namely, generation of inositol 1,3,4,5-tetrakisphosphate in conjunction with $InsP_3$ [10,12]. To dissociate $[Ca^{2+}]_i$ elevation from $InsP_3$ formation we employed TG, a blocker of the Ca^{2+} pump of the endoplasmic reticulum [20].

Our data suggest that in the RBL cells, TG induces a $[Ca^{2+}]_i$ increase by two distinct mechanisms (Fig. 5). Thus, experiments performed in media where free Ca^{2+} ion concentration is reduced, demonstrate that TG causes elevation of $[Ca^{2+}]_i$ originating from intracellular stores but it also induces a sustained Ca^{2+} influx from extracellular medium as was recently demonstrated for other cell types [24,25]. Both these processes seem to be a consequence of the inhibition of Ca^{2+} uptake by intracellular stores caused by TG.

To examine directly the effect of $[Ca^{2+}]_i$ elevation on Ca^{2+} influx, we measured $^{45}Ca^{2+}$ uptake by TG treated RBL cells. $^{45}Ca^{2+}$ uptake has been used to monitor changes in Ca^{2+} permeability of the plasma membrane, and initial uptake rates are generally assumed to represent the true undirectional Ca^{2+} influx into cells, probably unaffected by $[Ca^{2+}]_i$ [33]. However, extrusion of $^{45}Ca^{2+}$ from the cytosol by calcium pumps and exchangers tends to diminish the experimentally measured uptake, in particular, at longer measurements. Therefore, we buffered $[Ca^{2+}]_i$ by loading the RBL cells with Quin2, a high affinity chelator [32]. Indeed, one observes at the peak of the FcεRI-induced response, 3 min after optimal antigen stimulation, a net uptake of 4.4 ± 0.4 nmol/10^6 cells (Fig. 6), close to the values reported in earlier studies [32,34]. A higher Ca^{2+} uptake was, however, induced by TG (5.8 ± 0.2 nmol/10^6 cells 3 min after stimulation). Antigen and TG induced $^{45}Ca^{2+}$ uptake patterns are analogous to those observed for the respective $[Ca^{2+}]_i$ elevation they induce (Fig. 5). Thus, the maximal uptake and $[Ca^{2+}]_i$ levels were reached 60 s and 180 s after stimulation by FcεRI clustering and TG, respectively. The higher sustained levels of $[Ca^{2+}]_i$ induced by TG application, are further paralleled by the continued increase in $^{45}Ca^{2+}$ uptake. No direct action of TG causing a change in the cell-membranes Ca^{2+} permeability has so far been demonstrated and in fact, its capacity to act as a Ca^{2+} ionophore has been excluded [20,35]. Hence, the most likely rationale for the observed Ca^{2+} influx induced by TG is via opening of plasma membrane Ca^{2+} channels. These channels are apparently different from those described by Hoth and Penner [13] as these open at high $[Ca^{2+}]_i$. From the present data it cannot be resolved whether the emptying of intracellular Ca^{2+} stores precedes its influx. Most probably these two processes are both initiated very early after the TG application as demonstrated by the lower $[Ca^{2+}]_i$ peak of response in low Ca^{2+} medium (Fig. 5c). Alternatively, depletion of Ca^{2+} in the cells' medium by EGTA addition prior to TG, also causes a partial reduction of intracellular Ca^{2+} pools by e.g. efflux of Ca^{2+} ions to the extracellular medium.

The suppression of the FcεRI-induced $[Ca^{2+}]_i$ signal by prior addition of TG (Fig. 5b), suggests that both, TG and FcεRI-mediated stimuli recruit Ca^{2+} from com-

mon intracellular pool(s) (or from substantially overlapping ones). Their mode of action is different, however, as has been previously documented [6,17,20,23–25]. Thus, FcεRI clustering, that increases $[Ca^{2+}]_i$ in RBL cells through formation of $InsP_3$ [6,17], can no longer execute its mode of action while TG, that blocks the endoplasmic reticulum Ca^{2+}-ATPase, is present. However, the regularly observed peak levels of $[Ca^{2+}]_i$ are induced by TG irrespective of receptor clustering. Recent studies of the effects of TG on metacholin-stimulated parotid acinar cells [24], bradykinin-stimulated NG-115-401L neuronal cells [23] or CD2/CD3-stimulated lymphocytes [25] led to similar conclusions.

Application of Ca^{2+} ionophores or Ca^{2+} injections yield the non-physiologically high $[Ca^{2+}]_i$ levels of several micromolar. These perturbations were shown to be sufficient for causing secretion of mast cells [18,36]. However, as has been previously implied, a lower amplitude of increased $[Ca^{2+}]_i$ is not a sufficient stimulus for secretion [19]. Therefore, the observed increase in $[Ca^{2+}]_i$ caused by TG could, probably, be accounted for the secretory response, however low, in these cells (Figs. 1, 4c). Moreover, the synergistic secretory response to the combined stimuli by both, TG and antigen (Figs. 2, 4), could also be attributed to the TG enhanced increase of $[Ca^{2+}]_i$ above that elicitated by FcεRI clustering alone (Fig. 5b).

The present study revealed that the TG-induced changes in $[Ca^{2+}]_i$ of RBL cells, like the response to FcεRI-clustering, results from a combination of Ca^{2+} originating from internal stores and from influx. Since both these stimuli, apparently, affect the same internal Ca^{2+} pool(s), the results suggest that these pools' depletion might be sufficient to trigger the opening of plasma membrane channels. Activation of Ca^{2+} influx through these channels has been suggested in different cell types [4,11], to be closely related to the extent of depletion of intracellular Ca^{2+} pools. The recent demonstration by patch clamp technique that Ca^{2+} currents are apparently influenced by $[Ca^{2+}]_i$ is also in line with this hypothesis [13,37].

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