Surface Functions during Mitosis in Rat Basophilic Leukemia Cells

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ABSTRACT At the entry into mitosis, cells abruptly lose membrane activities such as phagocytosis, pinocytosis, and capping. The present studies test if mitotic cells also resist functional responses to cell surface ligand-receptor interactions. The IgE receptors of RBL-2H3 rat basophilic leukemia cells were labeled with anti-dinitrophenol IgE (anti-DNP-IgE) and then cross-linked with multivalent ligands (DNP-bovine serum albumin [BSA]; DNP-B-phycoerythrin; DNP-BSA-gold). IgE-receptor cross-linking modulates cell surface organization and function and releases serotonin and other mediators of allergic and asthmatic reactions from interphase cells (Pfeiffer, J. R., J. C. Seagrave, B. H. Davis, G. G. Deanin, and J. M. Oliver, 1985, J. Cell Biol., 101:2145–2155). It was found that anti-DNP-IgE-receptor complexes are preserved on the cell surface throughout mitosis; they continue to bind DNP-proteins, and the resulting antigen-IgE-receptor complexes can redistribute to coated pits on the cell surface. Furthermore, there is no loss of [3H]serotonin through mitosis. Nevertheless, antigen-stimulated [3H]-serotonin release is strongly impaired in mitotic-enriched as compared with mixed interphase or G1-enriched cell populations. In addition, antigen binding transforms the surface of interphase cells from a microvillous to a plicated topography and stimulates the uptake of fluorescein isothiocyanate-conjugated dextran by fluid pinocytosis. Mitotic cells maintain a microvillous surface topography after antigen treatment, and fluid pinocytosis virtually ceases from prometaphase to telophase. Phorbol myristate acetate, a tumor promoter that activates protein kinase C, restores surface ruffling activity to mitotic cells. Thus, the mitosis-specific freezing of membrane and secretory responses is most likely due to the failure of transmembrane signaling.

We have postulated that mitosis may represent a period when animal cells are specifically and reversibly insulated from environmental regulation. This hypothesis is based on the arrest in macrophages and other cells of a range of dynamic membrane functions (phagocytosis, pinocytosis, capping) at the onset of mitosis and their resumption at the entry of cells into G1 (22). The hypothesis predicts that cells should be incapable of response to hormones, growth factors, and immune effectors during mitosis. Consistent with this, Radley and Hodgson (26) have demonstrated that mitotic cells of the parotid and submaxillary glands are specifically resistant to isoprenaline-induced degranulation in vivo, Howard and Sheppard (14) have summarized and expanded the extensive evidence for low levels of cyclic AMP and correspondingly low activities of adenylyl cyclase during mitosis, and Preston et al. (25) have demonstrated that the adhesion and spreading of Cloudman M3 melanoma cells induced by melanocyte-stimulating hormone is inhibited during mitosis. Recently Hesketh et al. (13) reported that the IgE-mediated release of histamine from rat basophil leukemia cells is reduced during mitosis.

We report here further studies of the responses of mitotic RBL-2H3 cells to IgE-receptor cross-linking. RBL-2H3 cells were selected because (a) they can be synchronized (7) without use of anti-microtubule drugs that may disturb membrane functions, (b) their biochemical (1, 2, 19) and morphological (24) responses to cross-linking of cell surface IgE-receptor complexes with multivalent antigens have been well characterized, and (c) they have high density of IgE receptors (15) coupled with the recent development of new fluorescent antigens (10, 24) enables ready analysis of antigen-receptor binding, clus-
Fluorescence and Electron Microscopy: Cells were labeled with dinitrophenol-conjugated B-phycoerythrin (DNP-PhE), fluorescein isothiocyanate-conjugated dextran (fluorescein-dextran), and rhodamine-phalloidin as described (24). To identify mitotic cells, the nuclei of fixed cells were also labeled by incubation for an additional 10 min with 2 μg/ml Hoechst 33342, before rinsing, mounting, and observation by epillumination in a Zeiss photomicroscope III. Cells were processed for transmission and scanning electron microscopy as described (24).

Flow Cytometry: The efficiency of synchronization was followed by flow cytometry. Cells harvested by scraping at hourly intervals after removal of thymidine or hydroxyurea were fixed in 70% ethanol and their DNA labeled with propidium iodide. The amount of DNA per cell was measured using flow cytometry after labeling with propidium iodide. In B, the proportion of mitotic cells was determined by fluorescence microscopy after collecting the entire population by scraping or after collecting the mitotic-enriched cells by shake-off.

RESULTS

Synchronization

About 60% of thymidine-blocked cells are in early S phase as judged by flow cytometric analyses of their DNA content after labeling with propidium iodide. Upon removal of thymidine they progress in a fairly synchronous manner to G2 and mitosis (G2 + M) and finally to G1. The distribution of cells through these various phases with time after release of thymidine block is shown in Fig. 1 A. The presence of anti-DNP-IgE had no effect on the progression of synchronized cells through the cell cycle.

In cells released from thymidine block, mitotic shake-off yields populations that are 50–60% mitotic over a period of ~1 h (Fig. 1 B). Cell kinetics and mitotic yields of hydroxyurea-treated cells are closely similar to those of thymidine-blocked cells.

Mitotic cells can be assigned to one of five mitotic stages based on the distribution of the fluorescent dye Hoechst 33342: prophase, prometaphase, metaphase, anaphase, and telophase. The criteria for these assignments were developed previously (3), and most stages are illustrated in the micrographs that follow. Briefly, chromosomes are condensed and intercoiled and the nuclear envelope disassembles during telophase. The criteria for these assignments were developed previously (5), and most stages are illustrated in the micrographs that follow. Briefly, chromosomes are condensed and intercoiled and the nuclear envelope disassembles during telophase. The criteria for these assignments were developed previously (5), and most stages are illustrated in the micrographs that follow. Briefly, chromosomes are condensed and intercoiled and the nuclear envelope disassembles during telophase.
prophase; chromosomes become elongated, separated, and partially aligned during prometaphase; chromosomes assume a highly ordered, equatorial arrangement (the metaphase plate) at metaphase; at anaphase sister chromatids are separated and the cleavage furrow appears; and telophase cells show partly decondensed, widely separated chromosomes and a deep cleavage furrow. In synchronized RBL-2H3 ~60% of the mitotic harvest is metaphase cells, and the remaining cells are distributed between prometaphase, anaphase, and telophase. Cells in prophase are not detached by mitotic shake off. A high proportion of the contaminating interphase cells are G₁ pairs that have not yet reattached to the Blake bottles.

**Characteristic Properties of Mitotic RBL-2H3 Cells**

**DNP-PHE BINDING:** Fig. 2 shows that DNP-PhE binds at 4°C to both mitotic and interphase cells. Furthermore, microspectrophotometric measurements of DNP-PhE binding at 4°C revealed a small (~10%) but consistent increase in the relative fluorescence intensity of the mitotics as compared with cells judged to be in S or G₂ based on their large size and the high relative intensity of nuclear fluorescence due to Hoechst 33342. As expected, early G₁ cells, recognized by their small size and low relative intensity of Hoechst 33342 labeling, bound approximately half as much DNP-PhE as did the mitotics.

**[³H]SEROTONIN CONTENT:** The [³H]serotonin content per cell is highest in mitotic-enriched populations. In four experiments, mixed interphase populations contained ~80% as much [³H]serotonin per cell as the mitotics, and G₁-enriched populations contained ~60% as much [³H]serotonin per cell as the mitotic populations (Table I).

**Properties of Antigen-stimulated Mitotic Cells**

**[³H]SEROTONIN RELEASE:** In four experiments, binding of DNP-BSA and DNP-PhE to anti-DNP-IgE-receptor complexes stimulated the release of ~13% of the total [³H]serotonin content of G₁-enriched and mixed interphase cells in 15 min (Table I). Cell populations that were ~50% mitotic released only 7% of their total [³H]serotonin content in response to IgE-receptor cross-linking. These values are corrected for spontaneous [³H]serotonin release that remained approximately the same (between 4 and 6% [³H]serotonin release in 15 min) in mitotic-enriched, G₁-enriched, and mixed interphase cell populations.

In Table II the stimulated release of [³H]serotonin from mitotic cells is expressed as the fraction of stimulated [³H]serotonin release from G₁ and mixed interphase cells harvested from the same culture bottles. The data from four experiments were averaged and analyzed statistically using Student's t test. Cell populations that are ~50% mitotic release ~50% as much [³H]serotonin in response to IgE-receptor cross-linking as do the interphase cell populations. These differences are highly significant (P ≤ 0.005). There is no significant difference between G₁ and mixed interphase cells in stimulated [³H]serotonin release.

**SURFACE TOPOGRAPHY:** In the preceding paper the topography of unstimulated and antigen-stimulated RBL-2H3 cells was observed by scanning electron microscopy and by examination of rhodamine-phalloidin-labeled cells in the fluorescence microscope. It was shown that anti-DNP-IgE-primed RBL-2H3 cells have a characteristically microvillous
cell surface in the absence of antigen. Dinitrophenol-conjugated bovine serum albumin (DNP-BSA) treatment transforms the surface of interphase cells to a highly plicated topography.

The same analysis was performed here using mitotic-enriched populations. In the absence of antigen, both mitotic and interphase cells have a microvillous surface topography (Fig. 3A). Interphase cells are all plicated after incubation for

![Figure 3](image-url)

**Figure 3** Surface topography of mitotic cells: fluorescence microscopy. Suspensions of RBL-2H3 cells were incubated with or without DNP-BSA at 37°C for 10 min, then fixed and labeled with rhodamine-phalloidin and with Hoechst 33342. The cells were photographed to emphasize the topography of the upper cell surface. A 40X planapochromatic objective with partially closed diaphragm was used to minimize out-of-focus fluorescence at the cell periphery. In the absence of antigen, both interphase and mitotic (metaphase) cells have a microvillous cell surface (A and B). All the antigen-treated interphase cells show a plicated surface topography (C–F). The cells in prometaphase (C and D) and anaphase (E and F) are persistently microvillous. Bar, 10 μm.
FIGURE 4 Surface topography of mitotic cells: Scanning electron microscopy. Mitotic-enriched cell monolayers were incubated for 15 min in 1 μg/ml DNP-BSA, rinsed gently to minimize displacement of loosely adherent mitotic cells, and processed for scanning electron microscopy. Most cells show a plicated surface topography. The arrow in A points to a microvillous cell that is in early anaphase. The arrow in B points to a microvillous cell pair that is most likely in late anaphase. Other cells that are rounded and maintain a microvillous cell surface (cells on either side of the anaphase cell in A, for example) are also probably mitotic but this cannot be proven by scanning electron microscopy. Bars, 10 μm.

10 min with antigen (Fig. 3, C and E). However, the prometaphase cell in Fig. 4C and the anaphase cell in Fig. 3E maintain a persistently microvillous surface topography. Antigen-induced membrane plications were also observed by scanning electron microscopy on essentially all interphase cells, but never on cells judged from their shapes to be in anaphase or telophase (Fig. 4). Cells at earlier mitotic stages could not be identified with certainty by scanning electron microscopy.

Rhodamine-phalloidin-labeled cell populations were scored for their microvillous or plicated surface topography as a function of the mitotic cycle. Fig. 5A shows that cells in prophase and some prometaphase cells develop surface plications in response to antigen binding. Essentially all cells in metaphase and anaphase maintain a microvillous surface topography in the presence of antigen.

**FLUID PINOCYTOSIS:** Cross-linking of anti-DNP-IgE-receptor complexes by DNP-BSA on interphase cells stimulates the uptake of fluorescein–dextran by fluid pinocytosis (24). Mitotic cells between prometaphase and telophase internalize very little fluorescein–dextran in the presence or absence of DNP-BSA. This selective impairment of fluid uptake is illustrated in Fig. 6.

The results of population analyses of fluorescein–dextran-labeled cells are plotted as a bar graph in Fig. 7A. Fluid pinocytosis is reduced during prometaphase and is arrested in metaphase and anaphase.

**Dynamic Properties of Antigen–IgE–Receptor Complexes during Mitosis**

**INTERNALIZATION OF LIGAND–IGE–RECEPTOR COMPLEXES:** DNP-proteins bound to IgE-receptor complexes on interphase cells are rapidly redistributed to cytoplasmic vesicles that are visible by fluorescence microscopy of DNP-PhE–labeled cells and by transmission electron microscopy of DNP-BSA–gold–labeled cells (24). To determine if mitotics also internalize antigen, anti-DNP-IgE–primed, mitotic-enriched RBL-2H3 cells were incubated for 10 min at 37°C with DNP–PhE, followed by 2 min with the monovalent competing ligand, DNP–lysine, to displace most of the surface fluorescence. Intracellular DNP–PhE–IgE–receptor...
Fluid pinocytosis by interphase and mitotic cells. Anti-DNP-IgE-treated RBL-2H3 cells were incubated for 10 min at 37°C with Hanks'-BSA containing 1 µg/ml DNP-BSA and 20 mg/ml fluorescein-dextran (A). They were rinsed, fixed, and their DNA labeled with Hoechst 33342 (B). The interphase cells have all internalized fluorescein-dextran. There is no fluorescein-dextran uptake during metaphase (open arrows) and anaphase (closed arrows). Bar, 10 µm.

FLUORESCEIN-DEXTRAN UPTAKE

DNP-PHYCOERYTHRIN UPTAKE

STAGE OF MITOSIS

FIGURE 6 Fluoresent-dextran uptake and DNP-PhE uptake as a function of the mitotic cycle. IgE-primed cells were incubated for 10 min at 37°C in Hanks'-BSA containing 1 µg/ml DNP-BSA plus 20 mg/ml fluorescein-dextran (A) or with 1 µg/ml DNP-PhE (B). They were then fixed, and their DNA was labeled with Hoechst 33342. Mitotic and interphase cells were scored in the fluorescence microscope for the presence of intracellular vesicles containing fluorescein (A) or DNP-PhE (B). At least 100 cells were observed for each mitotic stage.

complexes were observed by fluorescence microscopy. DNP-PhE is internalized by most prometaphase cells (Fig. 8A) and by many metaphase cells (Fig. 8C). Anaphase cells internalize little or no antigen (Fig. 8E), but internalization resumes by telophase (Fig. 8G).

The results of population analyses of DNP-PhE-labeled cells are plotted in Fig. 7B. Almost all cells in prophase, prometaphase, and telophase can support the uptake of DNP-PhE-IgE-receptor complexes. However, only ~35% of metaphase cells internalize antigen, and only 15% of anaphase cells show uptake of DNP-PhE.

Redistribution of Antigen–IgE–Receptor Complexes to Coated Pits

DNP-BSA-gold particles are redistributed into coated pits on the cell surface of interphase RBL-2H3 cells before internalization. In the preceding paper (24) we showed that ~25% of surface-associated particles are in coated pits on interphase cells incubated for 2, 5, and 10 min with DNP-BSA-gold, whereas the proportion of intracellular gold particles increases steadily with time. These data indicate that a continuous process of binding, redistribution, and internalization of DNP-BSA–gold occurs during interphase.

Cells in mitosis also accumulate surface-bound gold particles in coated pits. Thus the anaphase cell in Fig. 9 was incubated for 10 min at 37°C with DNP-BSA–gold. 44 gold particles are bound and 37 (84%) of these particles are on the cell surface. The small number of intracellular particles is consistent with the reduction in antigen uptake established by fluorescence microscopy of anaphase cell. 12 (27%) of the surface-associated particles are in coated pits. The inset to Fig. 9 shows a portion of a metaphase (M) cell and a neighboring interphase (IP) cell from the same DNP-BSA-gold–labeled sample. The metaphase cell had 31 gold particles bound over its entire surface, of which 14 (45%) were in coated pits. The segment of the cell shown here has one coated pit that contains eight gold particles. Only one intracellular particle is visible. The interphase cell also has gold associated with a coated pit at the cell surface. In addition, numerous gold-containing cytoplasmic vacuoles are present. In a sample of 10 mitotic cells (six in prometa- or metaphase; four in ana- or telophase) that were similarly labeled for 10 min with DNP-protein–gold, no more than 50% of the total gold particles were even in the cytoplasm, and between 27% (the anaphase cell in Fig. 9) and 68% (a telophase cell) of surface-bound particles were in coated pits.

PMA Induces Surface Transformation in Mitotic Cells

The tumor promoter phorbol myristate acetate (PMA) was shown previously to transform the interphase cell surface from a microvillous to a plicated topography (24). Fig. 10 establishes that PMA can also transform the surface of mitotic cells. This transformation occurs on essentially every mitotic cell (Fig. 5B).

DISCUSSION

We set out to test the hypothesis that mitotic cells are specif-
FIGURE 8 The internalization of antigen during mitosis. Anti-DNP-IgE-treated cells were incubated for 10 min with 1 μg/ml DNP-PhE, followed by 2 min with 10 μM DNP-lysine. They were fixed and their nuclei labeled with Hoechst 33342. DNP-PhE is internalized by the cells in interphase (E and F), prometaphase (A and B), metaphase (C and D), and telophase (G and H). The cell in early anaphase (E and F) shows no intracellular fluorescence. Bar, 10 μm.

ically and reversibly resistant to activation by hormones, growth factors, and immune effectors that bind to membrane receptors on target cells. The RBL-2H3 cell line was selected for analysis for several reasons. First, the biochemical and morphological responses leading to the antigen-stimulated release of mediators ([3H]serotonin, histamine) from the secretory granules of interphase RBL-2H3 cells are fairly well defined. Briefly, antigen binding cross-links IgE-receptor complexes and leads, most likely via activation of a GTP-binding protein, to the increased hydrolysis of inositol phospholipids by phosphatidylinositol bisphosphate phosphodiesterase. This hydrolysis yields diacylglycerol that stimulates protein kinase C as well as inositol trisphosphate that mobilizes Ca²⁺ (1, 2, 9, 21). Antigen binding also causes an initial decrease followed by a marked increase in membrane-associated filamentous actin (24). These biochemical responses are accompanied by the transformation of membrane topography from microvillous to plicated, by an
The distribution of gold–DNP–BSA during mitosis. Mitotic-enriched anti-DNP-IgE-primed cells were incubated for 10 min with DNP–BSA (1 μg/ml). In the anaphase cell, surface-bound gold particles (circles) are associated with unspecialized membrane and with the membranes of coated pits (arrows). There are very few intracellular gold particles. Similarly the segment of a metaphase cell (inset) shows gold accumulated in a coated pit at the cell surface but only one intracellular particle. The inset also shows a segment of an interphase cell (IP) that has internalized a large number of gold particles. Bars, 1 μm.
increased rate of fluid pinocytosis, and by enhanced cell
spreading (24).

Second, each interphase cell has more than \(10^5\) IgE recep-
tors (16) and the mobility in the membrane and internaliza-
tion of IgE-receptor complexes can be followed by use of
fluorescent (8, 18, 24), radiolabeled (11, 16), and gold-conju-
gated (24) antigens, respectively.

Third, cell populations that are \(\sim 60\%\) mitotic can be ob-
tained by release from G1/S blocking agents (thymidine and
hydroxyurea) and subsequent mitotic shake-off. Higher yields
of mitotic cells are possible if nocodazole or colchicine are
used to prevent their progression beyond mitosis (13). How-
ever in our hands nocodazole and colchicine (0.1–10 \(\mu\)M; 1–
3 h) inhibit \(^{3}H\)serotonin release from interphase RBL-2H3
cells by 20–50\%. A similar inhibition of degranulation follows
microtubule disassembly in primary mast cells and basophils
(12, 27). In addition we have reported that anti-microtubule
drugs have different effects on the shape and membrane
properties of mitotic and interphase cells (4). Thus, a function
that seems unaltered by these drugs in interphase cells may
be altered during mitosis. For these reasons, we chose to use
imperfectly synchronized cells rather than infer normal mi-
totic properties from the responses of drug-treated cells.

We found that DNP-PhE binds to anti-DNP-IgE-receptor
complexes on mitotic cells, and that the intensity of fluores-
cence per mitotic cell is somewhat higher than the average
fluorescence intensity per interphase basophil. This indicates
that IgE receptors (and IgE-receptor complexes) are preserved
throughout mitosis. Similarly, Isersky et al. (15) reported that
IgE-receptor density on RBL-2H3 cells is high during mitosis
and is maximal during G1. Meyer et al. (20) confirmed a high
density of IgE receptors in G2 + M cells although they found
low receptor density on G1 cells. We also found that mitotic-
enriched populations have higher concentrations of \(^{3}H\)sero-
tonin than either mixed interphase or G1-enriched cells
harvested from the same culture bottles. These data indicate
that there is no loss of \(^{3}H\)serotonin to the medium during
the progression of unstimulated cells from S and G2 through
mitosis. There is of course a reduction in \(^{3}H\)serotonin con-
tent per cell in G1 as a result of the division of granules
between daughter cells at cytokinesis.

Even though IgE-receptor complexes and intracellular me-
diators are preserved throughout mitosis, anti-DNP-IgE-
treated RBL-2H3 cells release very little \(^{3}H\)serotonin when
incubated with DNP-proteins during mitosis. However cells
that bound IgE during S and G2 can release \(^{3}H\)serotonin in
response to antigen after progression to G1. Similarly, Hesketh
et al. (13) have reported that nocodazole-arrested mitotic RBL
cells fail to show antigen-IgE-dependent histamine release.
Thus, antigen-stimulated mediator release is specifically and
reversibly inhibited during mitosis.

Several other membrane responses to IgE-receptor cross-

![Figure 10](image_url)

**Figure 10** Surface transformation induced by PMA. Cells were incubated with 10 nM PMA, then labeled with rhodamine-
phalloidin (A and C) and Hoechst 33342 (B and D) and photographed to display the topography of the upper cell surface as
described in the legend to Fig. 4. The interphase cells, as well as the metaphase cell in A and B and the prometaphase cell in C
and D, are all extensively plicated. Bar, 10 \(\mu\)m.
linking are also impaired during mitosis in RBL-2H3 cells. In particular, interphase cells show a characteristic transformation of cell surface topography from a microvillous to a plicated appearance after antigen binding to IgE-receptor complexes (24). This surface response is arrested during prometaphase and is not restored until telophase. In addition, IgE-receptor cross-linking causes an immediate 2–3-fold stimulation of fluid pinocytosis by interphase cells. Like surface transformation, this response is substantially decreased in prophase/prometaphase, and fluid pinocytosis is not observed by fluorescence microscopy in metaphase and anaphase cells. The internalization of antigen per se is also reduced, although to a smaller extent than antigen-stimulated surface transformation and fluorescein–dextran uptake. Thus, the uptake of DNP–PhE into cytoplasmic fluorescent vesicles persists into metaphase and is strongly inhibited only in anaphase. Even anaphase cells can redistribute DNP–BSA–gold particles into coated pits, indicating that antigen–IgE–receptors retain at least some capacity for lateral movement through mitosis.

These results provide strong support for our starting hypothesis that signals produced by the interaction of specific ligand with surface receptors on target cells are either not generated or not recognized during mitosis.

How are mitotic cells isolated from environmental control? Warren and colleagues (13) reported that antigen binding to populations of nocardazole-arrested mitotic cells increase cytoplasmic free Ca\(^{2+}\) levels to ~50% of interphase levels. From this, they concluded that transmembrane signal generation is preserved during mitosis. They proposed that the impairment of cellular functions, including IgE-mediated release of histamine from RBL-2H3 cells, results from a generalized absence of membrane fusion and budding reactions during mitosis (13, 31). This hypothesis successfully predicts the impairment of both endocytic and exocytic processes during mitosis, as demonstrated in basophils and many other cell types (4–6, 13, 26, 28, 31, 32). However, it cannot easily explain why the transformation of RBL-2H3 cell surface topography from a microvillous to a plicated organization is inhibited during mitosis. Our previous demonstration that melanocyte-stimulating hormone fails to induce adhesion and spreading of mitotic melanoma cells (25) is also difficult to explain solely on the basis of the absence of membrane fusion and budding reactions.

Our data indicate that the failure of antigen-mediated cell responses during mitosis is indeed the result of impaired transmembrane signaling. The evidence for this comes from pharmacological studies. As noted, antigen binding normally stimulates the hydrolysis of phosphatidylinositol biphosphates, generating diacylglycerol that activates protein kinase C and inositol trisphosphate that stimulates Ca\(^{2+}\) mobilization. In the companion paper (24) we established that the membrane responses to IgE-receptor cross-linking are mimicked by PMA, the tumor promoter that directly stimulates protein kinase C-mediated phosphorylation reactions. We conclude that antigen-stimulated surface transformation is inhibited during mitosis due to a defect in the transduction pathway that leads from antigen binding to protein kinase C activation.

Ligand-stimulated Ca\(^{2+}\) mobilization is considered to be controlled by the same transduction pathway as ligand-stimulated protein kinase C activity (21). It is thus surprising that Warren and colleagues (13) found only a partial inhibition during mitosis of ligand-stimulated Ca\(^{2+}\) mobilization in Quin-2-labeled, nocardazole-arrested mitotic RBL-2H3 cells. An improved shake-off method to isolate 90% pure (or better) mitotic RBL-2H3 cells is being refined in our laboratory. We predict that A23187 plus Ca\(^{2+}\) will stimulate \(^{3}H\)serotonin release from these mitotic populations. We intend to re-examine antigen-stimulated Ca\(^{2+}\) uptake and free Ca\(^{2+}\) levels in these cells.

The improved mitotic populations will also simplify biochemical analyses of the signaling defect. We predict that changes in antigen binding affinity or in the kinetics of antigen-induced IgE–receptor cross-linking are responsible for the failure of signal transduction during mitosis. However the absence from the membrane or inactivation during mitosis of the GTP-binding protein (transducer) or phosphatidylinositol bisphosphate phosphodiesterase would also prevent transmembrane signaling in mitotic cells.

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