

Intracellular Elevations of Free Calcium Induced by Activation of Histamine H1 Receptors in Interphase and Mitotic HeLa Cells: Hormone Signal Transduction is Altered during Mitosis

Mario Volpi and Richard D. Berlin

Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032

Abstract. A broad range of membrane functions, including endocytosis and exocytosis, are strongly inhibited during mitosis. The underlying mechanisms are unclear, however, but will probably be important in relation to the mitotic cycle and the regulation of surface phenomena generally. A major unanswered question is whether membrane signal transduction is altered during mitosis; suppression of an intracellular calcium ($[Ca^{2+}]_i$) transient could inhibit exocytosis; $[Ca^{2+}]_i$ elevation could disassemble the mitotic spindle.

Activation of the histamine H1 receptor interphase in HeLa cells is shown here by Indo-1 fluorescence to produce a transient elevation of $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$ transient consists of an initial sharp rise that is at least partially dependent on intracellular calcium followed by an elevated plateau that is absolutely dependent

on extracellular calcium. The $[Ca^{2+}]_i$ transient is completely suppressed by preincubation with the tumor promoter, phorbol myristate acetate, but is unaffected by preincubation with pertussis toxin (islet-activating protein). In mitotic (metaphase-arrested) HeLa cells, the $[Ca^{2+}]_i$ transient is largely limited to the initial peak. Measurement of $^{45}Ca^{2+}$ uptake shows that it is stimulated by histamine in interphase cells, but not in mitotics. We conclude that the histamine-stimulated generation of the second messenger, $[Ca^{2+}]_i$, in mitotic cells is limited by failure to activate a sustained calcium influx. The initial phase of calcium mobilization from intracellular stores is comparable to that in interphase cells. Hormone signal transduction thus appears to be altered during mitosis.

A wide range of membrane functions is arrested during mitosis. These include endocytosis (1), transferrin receptor recycling (33, 42), and the transport of newly synthesized proteins (43) and glycosaminoglycans (29). A major unanswered question is whether hormone membrane signal transduction, including the generation of second messengers, is also altered in mitotic cells. Because AMP levels are generally depressed during mitosis (46), and because the mitotic spindle is disassembled by $[Ca^{2+}]_i$ (13), it seems likely that membrane events that lead to elevations of these second messengers are precisely controlled. Indeed, it now appears that a brief elevation of $[Ca^{2+}]_i$ is essential for nuclear envelope breakdown (37, 39) and is a normal accompaniment of the metaphase-anaphase transition (28). It is reasonable to suppose that the untimely elevation of $[Ca^{2+}]_i$ induced by hormones could adversely affect spindle function.

It is known that many of the consequences of hormonal stimulation are inhibited during mitosis. Thus, the isoproterenol-stimulated degranulation of salivary cells is specifically suppressed during mitosis (31), and, more recently, it has been shown that amine secretion by rat basophilic leukemia

cells in response to receptor binding of IgE-antigen complexes is also suppressed in mitotic cells (11, 26). These secretory processes, and other membrane functions described so far, share a requirement for membrane vesicle fusion, and Warren (41) has hypothesized that a defect in membrane-membrane fusion is the unifying mechanism underlying altered membrane functions of mitotic cells. However, the critical transduction events preceding membrane fusion have not been examined in detail. Thus, Hesketh et al. showed (11), using the Ca^{2+} indicator, Quin 2, that a rise in $[Ca^{2+}]_i$ did occur in mitotic basophilic leukemia cells that bound IgE-antigen complexes suggesting that signal transduction was not altered during mitosis, but the magnitude and duration of response were not precisely quantified.

Activation of the histamine H1 receptor of HeLa cells rapidly induces a membrane hyperpolarization (9) that is mediated by calcium-dependent potassium channels (35). The response declines even in the continued presence of histamine, and the cell becomes refractory to subsequent challenge by histamine. Histamine is known to induce a transient elevation of $[Ca^{2+}]_i$ in neuroblastoma cells (24) and smooth muscle (19). It was expected thus that histamine would induce a

[Ca²⁺]_i transient in HeLa cells. We show here that this is indeed the case and we use its characteristics to compare the responses of interphase and mitotic cells.

Materials and Methods

Cells

HeLa cells were grown in F-12 medium supplemented with L-glutamine and 7% FBS on tissue-culture plastic. After 3 d, exponentially growing cells were trypsinized and transferred to plastic Petri dishes on which they continued to grow without attachment. After 24 h, the cells were removed and dispersed by gentle aspiration into fresh medium.

Mitotic cells were obtained 15 h after addition of 0.1 µg/ml colcemid to monolayers. The loosely attached mitotic cells were collected by gentle shaking of the plates and concentrated by centrifugation. The mitotic index of the resuspended cells was ~90%. Within 1 h of removal of colcemid, the entire mitotic population progressed into G₁.

Measurement of [Ca²⁺]_i

[Ca²⁺]_i was measured using Indo-1, the fluorescent Ca²⁺ indicator, as described by Grynkiewicz et al. (8). Indo-1 shifts its emission spectrum with Ca²⁺ binding. The ratio of emission intensities at two wavelengths is thus related to Ca²⁺ independent of dye concentration. Fluorescence emission was monitored at 405 and 485 nm with excitation at 355 nm. The figures graph the [Ca²⁺]_i calculated according to the following formula:

$$[Ca^{2+}]_i = s \left(\frac{R - R_{min}}{R_{max} - R} \right) K_d,$$

in which K_d , the Ca²⁺-dye dissociation constant, was assumed to be 250 nM; s , the ratio of fluorescence at 485 nm of Indo-1 in the presence of EGTA to that in saturating calcium. R_{max} was determined after cytolysis in 0.1% NP-40 containing 0.5 mM Ca²⁺. 1 mM MnCl₂, which suppresses all fluorescence of the Indo-1, was added to the lysate to determine the background fluorescence of unhydrolyzed Indo-1 acetoxymethyl ester (AM)¹ (which is independent of [Ca²⁺]_i). The background was subtracted from the total fluorescence signal. This is essentially the method used by Hesketh et al. (11) and DiVirgilio et al. (5). The contribution of extracellular Indo-1, estimated by measurement of the supernatant fluorescence after pelleting the cells by centrifugation, equalled ~25 nM [Ca²⁺]_i and varied only slightly from experiment to experiment. It was ignored in the calculations.

2.2-ml aliquots of cell suspension, ranging from 0.3 to 0.6 × 10⁶ cells/ml, were incubated for 15 min with 1 µM of Indo-1 acetoxymethyl ester (Indo-1 AM) at 37°C, centrifuged and resuspended in 2 ml of warm conditioned medium. The cells were kept in suspension at 37°C by continuous stirring. By centrifuging the suspension at progressive intervals, it was determined that there was no dye leakage over the experimental time course (30 min) with or without histamine treatment. From the dye fluorescence obtained after detergent lysis of the cells, we estimated the dye content to have been ~10 pmol/10⁶ cells or a concentration of ~5–10 µM. Fluorescence measurements were carried out in an SLM-8000 spectrofluorimeter equipped with thermostatically controlled cuvette holder and magnetic stirrer.

Pertussis Toxin-mediated ADP-ribosylation

HeLa cells on Petri dishes were incubated 3 h in complete medium ± 100 ng/ml pertussis toxin (List Biological Laboratories, Campbell, CA). The control and pertussis toxin-treated cells were then collected, centrifuged down, washed once, and resuspended in 250 µl of ice-cold buffer of the following composition: 0.1 M sucrose; 5 mM Tris-HCl, pH 7.2; 2 mM EDTA; 0.25 mM diisopropylfluorophosphate (DFP); and 1 mM DTT. The cells were then disrupted by sonication by two 10-s exposures to the tip of a Kontes microultrasonic cell disrupter with the tune dial set on 5. The resulting homogenates were then tested for ADP-ribosylation using procedures modified from Okajima and Ui (25) and Goldman et al. (7). Samples were incubated for 30 min at 30°C in an assay medium containing: 87 mM su-

1. *Abbreviations used in this paper:* AM, acetoxymethyl ester; DFP, diisopropylfluorophosphate; DG, diacylglycerol; IP₃, inositol-tris-phosphate; NEBD, nuclear envelope breakdown; PIP, phosphoinositide-bis phosphate; PKC, protein kinase C.

crose; 4.3 mM Tris-HCl, pH 7.2; 0.22 mM DFP; 1 mM ATP; 12 µM NAD; 67 µCi/ml [α-³²P] NAD; 0.67 mM DTT; 6.7 mM K₂HPO₄; 10 mM thymidine; 0.67 mM EDTA; 2.5 mM MgCl₂, and, when present, 16.7 µg/ml pertussis toxin. Before addition to the mix, pertussis toxin was activated by 10 min incubation at 37°C in a solution of 100 mM potassium phosphate buffer, pH 7.5, containing 10 mM DTT.

Reactions were stopped by addition of SDS sample buffer (final concentration 3% SDS; 5% glycerol; 10 mM Tris-HCl; pH 7.8; 2% β-mercaptoethanol; 0.02% bromophenol blue). Samples were then boiled for 5 min and electrophoresed on an 11% acrylamide gel according to Laemmli (16). After electrophoresis, results were analyzed by exposing an XAR-5 Kodak film to the dry gel.

⁴⁵Ca²⁺ Uptake

Rapid measurements of ⁴⁵Ca²⁺ uptake were made by centrifuging cell suspensions through Versilube F-50 oil (20). Cells were resuspended in conditioned medium and incubated with gentle agitation for 15 min at 37°C. Aliquots of 0.4 ml containing ~4 × 10⁵ cells were pipetted onto Eppendorf centrifuge tubes containing 0.5 ml of Versilube F-50 oil previously equilibrated at 37°C. Histamine at a final concentration of 12 µM was rapidly added in a relatively large volume, usually 0.2 ml, to promote mixing, and the samples were allowed to remain at 37°C for an additional 2 min. 0.2 ml of conditioned medium containing 1.3 µCi of ⁴⁵Ca²⁺ were then added, giving an estimated specific activity of 4.3 µCi/µmol. Thus, uptake of ⁴⁵Ca²⁺ was studied during the plateau phase of the [Ca²⁺]_i transient. At the end of the incubation period, the cells were centrifuged through the oil, the aqueous medium and the oil were carefully removed, and the tip of the tube containing the pellet was cut off with a razor blade. The pellet was then dissolved with 0.5 ml of acetic acid, ACS (Amersham Corp., Arlington Heights, IL) was added, and the values of radioactivity were determined in a β counter.

Results

[Ca²⁺]_i in Interphase Cells

In interphase HeLa cells, histamine induced a dose-dependent increase in intracellular free calcium [Ca²⁺]_i (Fig. 1 A). Histamine concentrations >12 µM did not increase or prolong the response. The [Ca²⁺]_i transient was blocked by the specific H₁ antagonist, pyrilamine (0.1 µM), but not by the H₂ antagonist, cimetidine (0.1 mM) (not shown). In these experiments, the transient was generally characterized by a spike followed by a sustained plateau. In some instances, the [Ca²⁺]_i decreased gradually from its initial maximal elevation; no clear peak was apparent. Removal of extracellular [Ca²⁺]_i (Fig. 1 B) left a reduced spike with no sustained elevation. This suggested that the entire plateau phase and at least a part of the initial [Ca²⁺]_i rise were dependent on extracellular Ca²⁺. Only the initial elevation appeared dependent on mobilization of intracellular calcium stores.

The Histamine-induced [Ca²⁺]_i Transient is Blunted or Abolished by Phorbol Myristate Acetate (PMA)

Mobilization of intracellular calcium is likely to be mediated by (1, 4, 5) inositol-tris-phosphate (IP₃) (2). It is generated by cleavage of phosphoinositide-bis phosphate (PIP₂) with the concomitant production of (1,2)diacylglycerol (DG). DG is an activator of protein kinase C (PKC) and phorbol ester tumor promoters, which are potent surrogates of DG, activate PKC directly (36). The tumor promoter PMA has been shown in other systems to dampen the ligand receptor-induced calcium transients (e.g. DiVirgilio et al. [5]).

Preincubation of cells for as little as 2 min with 100 nM PMA almost completely blocked the histamine-induced response (Fig. 1 C). The nontumor promoter isomer 4-α-phorbol-12,13-didecanoate at 1.6 µM had no effect (not shown).

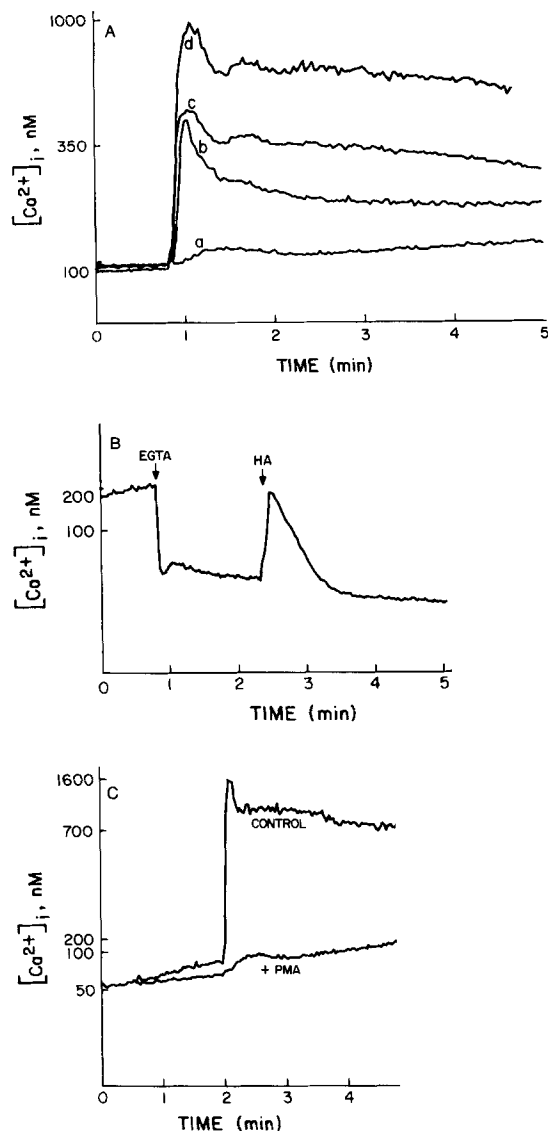


Figure 1. Intracellular free calcium transients induced by histamine in suspensions of interphase HeLa cells. (A) The histamine concentrations corresponding to the curves (a–d) were: (a) 0.05 μ M, (b) 2.0 μ M, (c) 6.0 μ M, and (d) 12.0 μ M. The general pattern of the $[Ca^{2+}]_i$ transient is illustrated in this representative experiment: a sharp peak followed by a high plateau. (B) Effect of chelation of extracellular Ca^{2+} on the histamine-induced $[Ca^{2+}]_i$ transient in interphase HeLa cells. 3 mM EGTA, and 12 μ M histamine (HA), were added at the arrows. (C) Effect of preincubation of interphase HeLa cells with PMA on the $[Ca^{2+}]_i$ transient. 100 nM PMA was added at zero time. 2 min later, 10 μ M histamine was injected to PMA-treated and control samples.

The HeLa HI system thus is different from that in rat hepatocytes, in which PMA did not inhibit the effect of histamine on metabolic pathways (6). ($[Ca^{2+}]_i$ was not measured in their experiments.)

Pertussis Toxin Does Not Affect the Histamine Response

Since calcium mobilization by some hormones appears to be transduced by membrane-associated GTP binding proteins (G proteins) that are blocked by pertussis toxin, we asked if

preincubation of intact HeLa cells with pertussis toxin affected the $[Ca^{2+}]_i$ transient. Fig. 2 A shows that such preincubation did not affect the transient. It was possible, however, that in intact cells the toxin had not gained access to its substrate. Further studies showed this was not the case.

Pertussis toxin effects the ADP-ribosylation of the 40-kD α subunit of the G_i/G_o oligomer (14, 22). We determined if this had occurred during preincubation of cells with the toxin by examining the extent of ADP-ribosylation that could be catalyzed in homogenates: if the ADP-ribosylation site were saturated, no further conjugation would be possible. Fig. 2 B shows that pertussis toxin readily catalyzed the incorporation of label from $[^{32}P]NAD$ to a 40-kD protein in homogenates of control cells (lane a'), but not in homogenates of cells that had been treated with toxin (*b'*). The absence of a pertussis toxin effect clearly distinguishes the histamine response of HeLa cells from *Aplysia*, in which a G protein pertussis toxin substrate appears to regulate the opening of K^+ -channels (34), but is similar to the HI system described by Nakahata in astrocytoma cells (21) and to the muscarinic system described by Masters et al. (18).

Rapid Downregulation of the Calcium Response

If cells were stimulated with as little as 50 nM of histamine and then histamine was added at concentrations up to 1–10 μ M, no increase in $[Ca^{2+}]_i$ was observed (not shown).

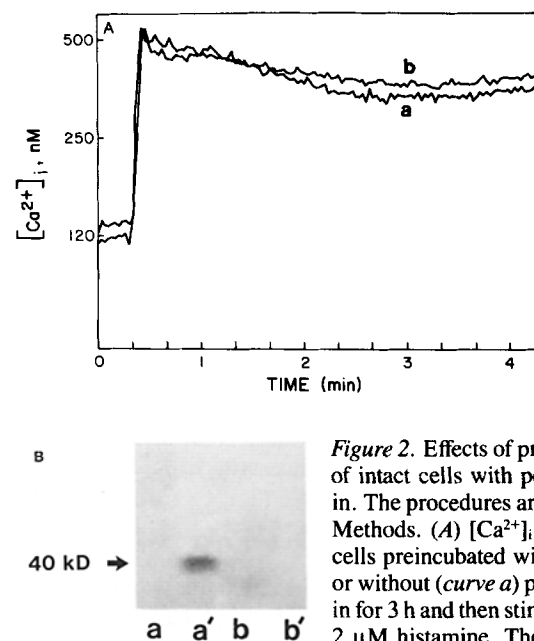


Figure 2. Effects of preincubation of intact cells with pertussis toxin. The procedures are detailed in Methods. (A) $[Ca^{2+}]_i$ transient in cells preincubated with (curve b) or without (curve a) pertussis toxin for 3 h and then stimulated with 2 μ M histamine. The results are essentially indistinguishable. It was hypothesized that any pertussis toxin effect would be more visible at this lower histamine dose. Effects were also not apparent at 12 μ M histamine. (B) Evidence for ADP-ribosylation of pertussis toxin substrate during incubation of intact cells: a 40-kD membrane protein that is ADP-ribosylated by pertussis toxin in homogenates of control cells not ADP-ribosylated in homogenates of cells that had been preincubated with pertussis toxin. Lanes a and a': control cells (not preincubated with pertussis toxin). Lane a homogenate, no toxin added to homogenate; lane a' homogenate, toxin added after homogenization. Lanes b and b': cells preincubated with 100 nM pertussis toxin. Lane b homogenate, no toxin added to homogenate; lane b' homogenate, toxin added after homogenization.

Hazama et al., in their report of the effects of histamine on the membrane potential of HeLa cells, also found a rapid loss of responsiveness (9).

$[Ca^{2+}]_i$ in Mitotic Cells

Armed with this characterization of the $[Ca^{2+}]_i$ transient in interphase cells, we examined histamine membrane signal transduction during mitosis. Fig. 3 A shows the $[Ca^{2+}]_i$ transients induced by histamine in a population of metaphase-arrested mitotic cells. In general, the peak heights of $[Ca^{2+}]_i$ attained by mitotic cells were very similar to those of interphase cells. In interphase cells the peak height was 976 ± 195 (SE) nM (11 experiments) and in mitotics, 797 ± 130 (SE) nM (7 experiments). Statistically these are not significantly different. Interestingly the basal (control) values were for interphase, 115 ± 12 (SE) (25 experiments), and for mitotics, 53 ± 14 (SE) (18 experiments) and were statistically different, $P < 0.001$. A similar difference between interphase and metaphase kidney epithelial cells was observed by Ratan et al. (32), whereas Poenie et al. (28) found comparable but somewhat elevated levels.

The half-maximal response of the peak was between 2 and 5 μ M histamine in both interphase and mitotic cells. However, the plateau of $[Ca^{2+}]_i$ manifested by interphase cells was markedly reduced in mitotics. Even the small residual plateau may be attributed at least in part to the presence of $\sim 10\%$ interphase cells present in the mitotic population.

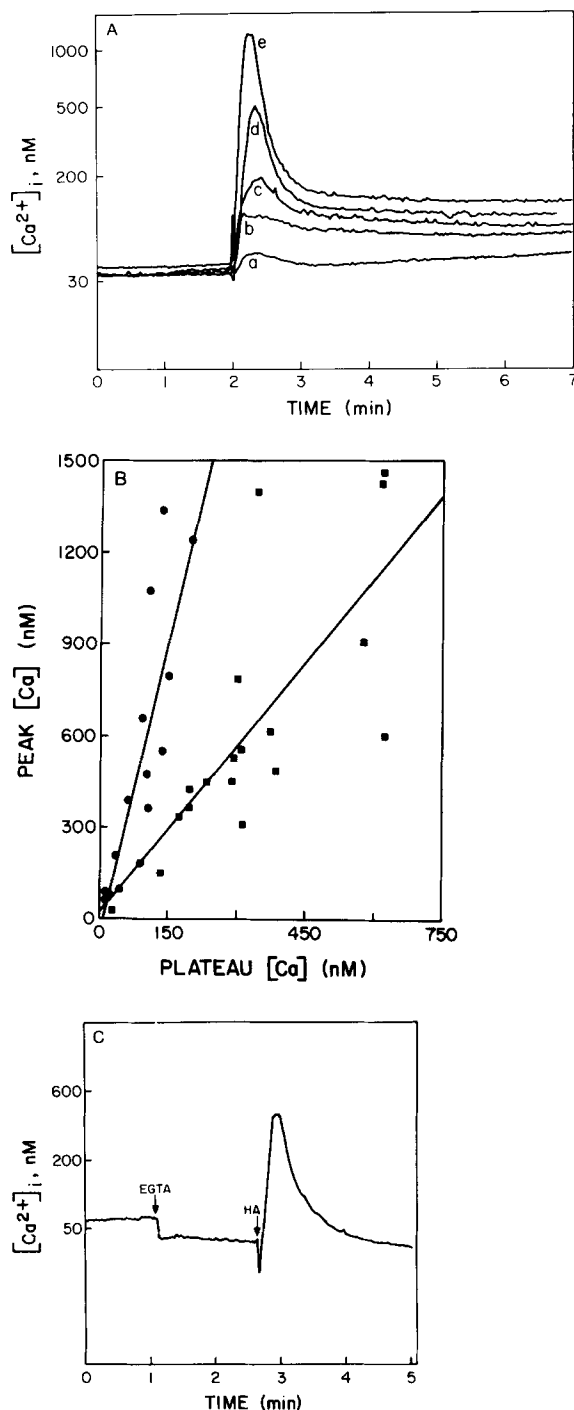
Addition of 0.1 μ M colcemid, the concentration used to obtain metaphase arrest, to interphase HeLa cells for 3 h (microtubules were completely disassembled by 30 min; not shown) had no effect on the $[Ca^{2+}]_i$ transient.

To quantify this difference in the shape of the $[Ca^{2+}]_i$ transient and to assess whether the peak height was a determinant of the plateau, we plotted the increment of $[Ca^{2+}]_i$ effected by various histamine concentrations at the peak, versus the increment of $[Ca^{2+}]_i$ 2 min after the addition of histamine (plateau) for 15 experiments with mitotic cells and 18 with interphase (Fig. 3 B). It is seen that after the same rise in $[Ca^{2+}]_i$ at the peak, the $[Ca^{2+}]_i$ increment at the plateau is consistently much lower in mitotics (*discs*) as compared with interphase (*squares*). Indeed, from the slope of the line (fitted by least squares) that essentially goes through the origin, the ratios of the change in $[Ca^{2+}]_i$ at the peak to that at the plateau were 6.5 for mitotic cells and 1.8 for interphase cells. In mitotics, total $[Ca^{2+}]_i$ did not exceed 200 nM during the plateau at any histamine dosage.

As for interphase cells, the $[Ca^{2+}]_i$ transient in mitotics was completely suppressed by preincubation with PMA. Preincubation with pertussis toxin also effected the ADP-ribosylation of a 40-kD protein, but as for interphase cells, did not alter the $[Ca^{2+}]_i$ transient.

Because the plateau in interphase cells was shown to be dependent on extracellular Ca^{2+} (Fig. 1 B), it was expected

Figure 3. Histamine-induced $[Ca^{2+}]_i$ transients in mitotic HeLa cells. Suspensions of metaphase-arrested HeLa cells were loaded with Indo-1 as described for interphase cells (Fig. 1), and the ratio of fluorescence emission (405/485 nm) determined. (A) Dose dependence of $[Ca^{2+}]_i$ response of mitotic cells. The histamine (HA) concentrations corresponding to each curve (a-e) were as follows: (a) 0.05 μ M; (b) 0.5 μ M; (c) 2.0 μ M; (d) 6.0 μ M; and (e) 12 μ M.



(B) Relationship of peak to plateau $[Ca^{2+}]_i$ after stimulation of mitotic or interphase HeLa cells with histamine. $[Ca^{2+}]_i$ was calculated as described in Methods. Basal (control) $[Ca^{2+}]_i$ levels were subtracted from the total $[Ca^{2+}]_i$ at the peak (peak $[Ca]$) and at 2 min after the addition of histamine (plateau $[Ca^{2+}]_i$). (●) mitotic cells; (■) interphase cells. The range of $[Ca^{2+}]_i$ was obtained by stimulation with varying the concentration of histamine. There was somewhat greater variability of the response of interphase cells, but there is almost no overlap with the data obtained from mitotic cells. The lines are drawn by least squares fit: the correlation coefficient, $r = 0.82$ (mitotics); $r = 0.76$ (interphase): The slope of the line for mitotics is approximately four times that for interphase cells. (C) Effect of chelation of extracellular Ca^{2+} on response of mitotic cells. 3 mM EGTA and 12 μ M histamine (HA) were added at the arrows.

that the $[Ca^{2+}]_i$ transient in mitotic cells with its reduced plateau would be only slightly affected by removal of extracellular Ca^{2+} . Indeed, the mitotic $[Ca^{2+}]_i$ transient was relatively unaffected when extracellular Ca^{2+} was chelated (Fig. 3 C); compare Fig. 1, A and B. It thus was suggested that the difference in $[Ca^{2+}]_i$ transient between mitotic and interphase HeLa cells depended on the rate of histamine-stimulated Ca^{2+} influx.

Uptake of $^{45}Ca^{2+}$

To examine the influx directly, we measured the uptake of $^{45}Ca^{2+}$ by suspensions of interphase or mitotic cells under conditions that were identical to those used to measure the $[Ca^{2+}]_i$ transient. Fig. 4 illustrates a representative experiment (one of three) showing the time course of uptake. To focus on events during the plateau in which the $[Ca^{2+}]_i$ elevation appeared to be dependent entirely on extracellular calcium, $^{45}Ca^{2+}$ was added 2 min after the addition of histamine. The nonzero ordinate at time zero corresponded to the amount of $^{45}Ca^{2+}$ trapped in the pellet. Uptake by interphase cells (*solid lines*) was sharply stimulated by histamine. Uptake by mitotic cells (*dashed lines*) was actually depressed slightly by histamine in this experiment.

Discussion

These results establish first that in HeLa cells, histamine, via H1 receptors, induces a rise in $[Ca^{2+}]_i$. The rise consists of an initial elevation that is at least partially due to mobilization of intracellular stores, and a longer sustained elevation that depends entirely on the availability and accelerated uptake of extracellular Ca^{2+} . Presumably this rise would activate the K^+ -channels described by Sauve et al. (35) that in turn causes a membrane hyperpolarization (9).

The histamine-induced $[Ca^{2+}]_i$ transient is qualitatively and quantitatively different in mitotic as compared with interphase HeLa cells. The initial elevation of $[Ca^{2+}]_i$ is similar, but the longer sustained elevation that depends on uptake of extracellular Ca^{2+} is sharply diminished in mitotics. IP_3 levels are increased by activation of H1 receptors in smooth

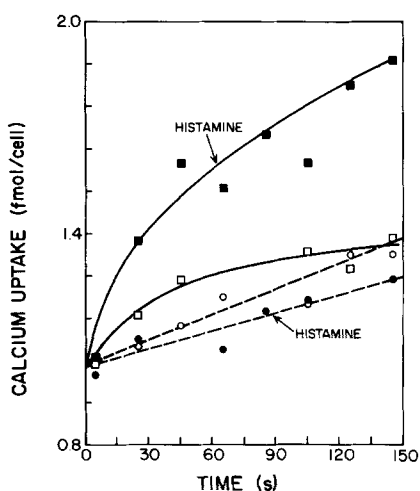


Figure 4. $^{45}Ca^{2+}$ influx in interphase and mitotic cells. —, interphase; - - -, mitotics; open symbols, unstimulated (controls); closed symbols, 12 μM histamine.

muscle (3), and in other systems appear to mediate Ca^{2+} release from intracellular stores (2). We assume that IP_3 is at least one of the mediators of the initial Ca^{2+} elevation observed in our study. The mechanisms controlling Ca^{2+} influx, on the other hand, are more obscure. IP_3 , acting through some special compartment that abuts plasma membrane, has been suggested as a possible mediator of Ca^{2+} influx in non-excitable cells (e.g. reference 30), and Kuno and Gardner (15) have shown that IP_3 can directly activate calcium channels in membrane patches excised from human T lymphocytes. The specific reduction of this phase of the histamine response in mitotic cells may provide a useful model for analysis of the influx mechanism.

It is possible that differences in interphase and mitotic H1 receptors contribute to the observed differences in $[Ca^{2+}]_i$ transient. The number of surface H1 receptors that would be available to exogenous histamine is unclear, both because the measurement of surface H1 receptors is problematical and because histamine as a univalent cation may have access to intracellular receptors. However, several observations indicate that the kinetic properties of the receptor are not the basis for the observed differences in interphase and mitotic cells. First, the concentration at which 50% of maximal release was seen, was between 2 and 5 μM for both interphase and mitotic cells; second, the minimum dose that consistently yielded a response, 50 nM, and the dose required for a maximum response, 12 μM , were the same for both. Most importantly, as shown in Fig. 3, at the same peak $[Ca^{2+}]_i$, the plateau is markedly depressed in mitotics.

The rapid downregulation of the H1 response may be explained through activation of PKC by DG (the byproduct of phosphoinositide bis-phosphate cleavage by phospholipase C that yields IP_3) (2). This is strongly suggested by the suppression of the $[Ca^{2+}]_i$ transient by the DG surrogate, PMA (Fig. 1 C). It should be emphasized, however, that both phases of the $[Ca^{2+}]_i$ transient are suppressed by PMA, and hence, PKC activation is unlikely to explain differences in the $[Ca^{2+}]_i$ transient between interphase and mitotic cells. This needs more careful examination. There appears to be a specific increase in phosphorylation of several proteins during mitosis (27, 44).

What is the mechanism underlying the specific inhibition of histamine-stimulated Ca^{2+} influx in mitotic cells? There are three possibilities: (a) the generation of IP_3 or another intermediate that activates the Ca^{2+} transporters, presumably Ca channels, is inhibited; (b) the channels are biochemically inactivated; or (c) they are physically removed from the surface. That the peak $[Ca^{2+}]_i$ is comparable for interphase and mitotics suggests that at least the initial generation of IP_3 and the mobilization of intracellular calcium stores are not affected. Precedents exist for both inactivation and removal mechanisms. Inactivation or inhibition of calcium channels by phosphorylation, or interaction with G proteins is well established (12). On the other hand, the selective removal of surface constituents during mitosis has also been described. Warren et al. showed this for transferrin receptors, which are internalized during prophase and metaphase and subsequently recycled to the cell surface (42). There is also limited precedent for the insertion of ion channels and transport systems via exocytosis of membrane vesicles. The proton-secreting membrane ATPase of turtle bladder (4, 40) and sodium channels of the rabbit urinary bladder (17) ap-

pear to be inserted into the apical membrane of their epithelia in this way. Neither mechanism is established by our experiments. However, there are important limitations to regulation by internalization of calcium channels that, in our opinion, make it a less likely mechanism. An internalization of channels within vesicle membrane would involve the concomitant internalization of extracellular calcium. Such channels would still be exposed to the same presumed cytoplasmic factors generated by H₁ receptor activation that in interphase cells induce calcium influx from extracellular fluid. An influx from the vesicle store would then occur down the same electrochemical gradient that affects influx from the extracellular medium. Note that vesicles would contain calcium at its extracellular concentration, here ~400 μM, whereas [Ca²⁺]_i is ~0.1 μM. The magnitude of this concentration difference also makes it unlikely that the content of vesicle calcium would be limiting. Obviously, other regulatory mechanisms could be superimposed on the internalized channel, but in its simplest form, a selective internalization of calcium channels during mitosis appears unlikely.

In any event, our results indicate clearly that signal transduction is altered during mitosis. Failure to induce the calcium influx required for a sustained elevation of [Ca²⁺]_i is in itself likely to inhibit secretory and other surface phenomena. For example, Neher and Almers showed that fast calcium transients were insufficient to trigger exocytosis by mast cells (23), and Truett et al. found that only sustained elevations of [Ca²⁺]_i supported the respiratory burst of neutrophils in response to chemoattractants (38). Note that the duration of the [Ca²⁺]_i peak in mitotic cells is the sum of a cell population whose individuals almost certainly express still briefer peaks.

On the other hand, the stimulation of even a transient elevation of [Ca²⁺]_i could have important consequences for the function of the mitotic spindle. It is generally held that [Ca²⁺]_i plays a critical regulatory role in mitosis (e.g., references 13, 28, 32, and 45). As noted above, an apparent rise in [Ca²⁺]_i precedes nuclear envelope breakdown (NEBD) and anaphase in several cell types, and this rise before NEBD has been recently shown to be essential for its occurrence (37, 39). The timing of these elevations of [Ca²⁺]_i appears crucial. In early embryos of the sea urchin, NEBD can be triggered prematurely by injection of calcium (37). Microinjection of calcium into the metaphase spindle at low concentrations advances entry into anaphase, whereas buffering the [Ca²⁺]_i delays entry. Similarly, lowering extracellular calcium prolongs metaphase in *Tradescantia*; an influx of calcium appears essential for the splitting of sister chromosomes (10). It is thus reasonable to expect that histamine-induced [Ca²⁺]_i transients could affect the timing of the mitotic cycle or perhaps even spindle structure. Indeed, histamine, as described, could be used to introduce discrete elevations of [Ca²⁺]_i to help determine the role of calcium transients in mitosis.

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References

1. Berlin, R. D., J. M. Oliver, and R. F. Walter. 1978. Surface functions dur-

- ing mitosis 1: phagocytosis, pinocytosis and mobility of surface-bound Con A. *Cell*. 15:327-341.
2. Berridge, M. J. 1984. Inositol trisphosphate and diacylglycerol as second messengers. *Biochem. J.* 220:345-360.
3. Bielikiewicz-Vollrath, B., J. R. Carpenter, R. Schulz, and D. A. Cook. 1987. Early production of 1,4,5-inositol trisphosphate and 1,3,4,5-inositol tetrakisphosphate by histamine and carbachol in ilial smooth muscle. *Mol. Pharmacol.* 31:513-522.
4. Cannon, C., J. vanAdelsberg, S. Kelly, and Q. Al-Awqati. 1985. Carbon-dioxide-induced exocytotic insertion of H⁺ pumps in turtle-bladder luminal membrane: role of cell pH and calcium. *Nature (Lond.)*. 314:443-446.
5. DiVirgilio, F., B. C. Meyer, S. Greenberg, and S. C. Silverstein. 1988. Fc receptor-mediated phagocytosis occurs in macrophages at exceedingly low cytosolic Ca²⁺ levels. *J. Cell Biol.* 106:657-666.
6. Garcia-Sainz, J. A., M. C. De la Garza, J. L. Contreras-Rodriguez, and A. Najhera-Alvarado. 1987. Effects of histamine on the metabolism of isolated rat hepatocytes: Roles of H₁ and H₂-histamine receptors. *Mol. Pharmacol.* 31:253-258.
7. Goldman, D. W., F.-H. Chang, L. A. Gifford, E. J. Goetzl, and H. R. Bourne. 1985. Pertussis toxin inhibition of chemotactic factor-induced calcium mobilization and function in human polymorphonuclear leukocytes. *J. Exp. Med.* 162:145-156.
8. Grynkiewicz, G., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440-3450.
9. Hazama, A., T. Yada, and Y. Okada. 1985. HeLa cells have histamine H₁-receptors which mediate activation of the K⁺ conductance. *Biochim. Biophys. Acta.* 845:249-253.
10. Hepler, P. K. 1985. Calcium restriction prolongs metaphase in dividing *Tradescantia* stamen hair cells. *J. Cell Biol.* 100:1363-1368.
11. Hesketh, T. R., M. A. Beaver, J. Rogers, B. Burke, and G. B. Warren. 1984. Stimulated release of histamine by a rat mast cell line is inhibited during mitosis. *J. Cell Biol.* 98:2250-2254.
12. Hofmann, F., W. Nastainczyk, A. Rohrkasten, T. Schneider, and M. Sieber. 1987. Regulation of the L-type calcium channel. *Trends Pharmacol. Sci.* 8:393-398.
13. Izant, J. G. 1983. The role of calcium ions during mitosis. Calcium participates in the anaphase trigger. *Chromosoma*. 88:1-10.
14. Katada, T., and Ui, M. 1982. ADP-ribosylation of the specific membrane protein of C6 cells by islet-activating protein associated with modification of adenylate cyclase activity. *J. Biol. Chem.* 257:7210-7216.
15. Kuno, M., and P. Gardner. 1987. Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. *Nature (Lond.)*. 326:301-304.
16. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680-685.
17. Lewis, S. A., and J. L. C. deMoura. 1984. Apical membrane area of rabbit urinary bladder increases by fusion of intracellular vesicles: an electrophysiological study. *J. Membr. Biol.* 82:123-136.
18. Masters, S. B., M. W. Martin, T. K. Harden, and J. H. Brown. 1985. Pertussis toxin does not inhibit muscarinic-receptor-mediated phosphoinositide hydrolysis or calcium mobilization. *Biochem. J.* 227:933-937.
19. Matsumoto, T., H. Kanaide, J. Nishimura, Y. Shogakiuchi, S. Kobayashi, and M. Nakamura. 1986. Histamine activates H₁-receptors to induce cytosolic free calcium transients in cultured vascular smooth muscle cells from rat aorta. *Biochem. Biophys. Res. Commun.* 135:172-177.
20. Melmed, R. N., P. J. Karanian, and R. D. Berlin. 1981. Control of cell volume in the J774 macrophage by microtubule disassembly and cyclic AMP. *J. Cell Biol.* 90:761-768.
21. Nakahata, N., M. W. Martin, A. R. Hughes, J. R. Hepler, and T. K. Harden. 1986. H₁-histamine receptors on human astrocytomas cells. *Mol. Pharmacol.* 29:188-195.
22. Neer, E. J., J. M. Lok, and L. G. Wolf. 1984. Purification and properties of the inhibitory guanine nucleotide regulatory unit of brain adenylate cyclase. *J. Biol. Chem.* 259:14222-14229.
23. Neher, E., and W. Almers. 1986. Fast calcium transients in rat peritoneal mast cells are not sufficient to trigger exocytosis. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:51-53.
24. Ohsako, S., and T. Deguchi. 1984. Receptor-mediated regulation of calcium mobilization and cyclic GMP synthesis in neuroblastoma cells. *Biochem. Biophys. Res. Commun.* 122:333-339.
25. Okajima, F., and M. Ui. 1984. ADP-Ribosylation of the specific membrane protein by islet-activating protein, pertussis toxin, associated with inhibition of a chemotactic peptide-induced arachidonate release in neutrophils. *J. Biol. Chem.* 259:13863-13871.
26. Oliver, J. M., J. C. Seagrave, J. R. Pfeiffer, M. L. Feibig, and G. G. Deanin. 1985. Surface functions during mitosis in rat basophilic leukemia cells. *J. Cell Biol.* 101:2156-2166.
27. Ottaviano, Y., and L. Gerace. 1985. Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Biol. Chem.* 260:624-632.
28. Poenie, M., J. Alderton, R. Steinhardt, and R. Tsien. 1986. Calcium rises abruptly and briefly throughout the cell at the onset of anaphase. *Science (Wash. DC)*. 233:886-889.
29. Preston, S. F., C. S. Regula, P. R. Sager, C. B. Pearson, L. S. Daniels, P. A. Brown, and R. D. Berlin. 1985. Glycosaminoglycan synthesis in-

- depressed during mitosis and elevated during early G1. *J. Cell Biol.* 101: 1086–1093.
30. Putney, J. W., Jr. 1986. A model for receptor-regulated calcium entry. *Cell Calcium.* 7:1–12.
 31. Radley, J. M., and G. S. Hodgson. 1971. Effect of isoprenaline on cells in different phases of the mitotic cycle. *Exp. Cell Res.* 69:148–160.
 32. Ratan, R. R., M. L. Shelarski, and F. R. Maxfield. 1986. Transition from metaphase to anaphase is accompanied by local changes in cytoplasmic free calcium in PtK2 kidney epithelial cells. *Proc. Natl. Acad. Sci. USA.* 83:5136–5140.
 33. Sager, P. R., P. A. Brown, and R. D. Berlin. 1984. Analysis of transferrin recycling in mitotic and interphase HeLa cells by quantitative fluorescence microscopy. *Cell.* 39:275–282.
 34. Sasaki, K., and M. Sato. 1987. A single GTP-binding protein regulates K⁺-channels coupled with dopamine, histamine, and acetylcholine receptors. *Nature (Lond.).* 325:259–262.
 35. Sauve, R., C. Simoneau, L. Parent, R. Monette, and G. Roy. 1987. Oscillatory activation of calcium-dependent potassium channels in HeLa cells induced by histamine H₁ receptor stimulation: a single-channel study. *J. Membr. Biol.* 96:199–208.
 36. Sharkey, N. A., K. L. Leach, and P. M. Blumberg. 1984. Competitive inhibition by diacylglycerol of specific ester binding. *Proc. Natl. Acad. Sci. USA.* 81:607–610.
 37. Steinhardt, R. A., and J. Alderton. 1988. Intracellular free calcium rise triggers nuclear envelope breakdown in the sea urchin embryo. *Nature (Lond.).* 332:364–366.
 38. Truett, A. P. III, M. W. Verghese, S. B. Dillon, and R. Snyderman. 1988. Calcium influx stimulates a second pathway for sustained diacylglycerol production in leukocytes activated by chemoattractants. *Proc. Natl. Acad. Sci. USA.* 85:1549–1553.
 39. Twigg, J., R. Patel, and M. Whitaker. 1988. Translational control of InP₃-induced chromatin condensation during the early cell cycles of sea urchin embryos. *Nature (Lond.).* 332:366–369.
 40. VanAdelsberg, J., and Q. Al-Awqati. 1986. Regulation of cell pH by Ca²⁺-mediated exocytotic insertion of H⁺-ATPase. *J. Cell Biol.* 102: 1638–1645.
 41. Warren, G. 1985. Membrane traffic and organelle division. *Trends Biochem. Sci.* 10:439–443.
 42. Warren, G., J. Davoust, and A. Cockcroft. 1985. Recycling of transferrin receptors in A431 cells is inhibited during mitosis. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2217–2225.
 43. Warren, G., C. Featherstone, G. Griffiths, and B. J. Burke. 1983. Newly synthesized G proteins of vesicular stomatitis virus is not transported to the cell surface during mitosis. *J. Cell Biol.* 97:1623–1628.
 44. Westwood, J. T., R. B. Church, and E. B. Wagenaar. 1985. Changes in protein phosphorylation during the cell cycle of Chinese hamster ovary cells. *J. Biol. Chem.* 260:10308–10313.
 45. Wolniak, S. M., and K. M. Bart. 1986. Nifedipine reversibly arrests mitosis in stamen hair cells of *Tradescantia*. *Eur. J. Cell Biol.* 33:273–277.
 46. Zeilig, C. E., R. A. Johnson, E. W. Sutherland, and D. L. Friedman. 1976. Adenosine 3':5'-monophosphate content and actions in the division cycle of synchronized HeLa cells. *J. Cell Biol.* 71:515–534.