Hyperosmotic modulation of the cytosolic calcium concentration in a rat osteoblast-like cell line

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1. The effects of hyperosmotic stress on cytosolic calcium concentration ($[Ca^{2+}]_i$) were studied by ratio image analysis in single cells of an osteoblast-like bone cell line (RCJ 1.20) loaded with fura-2 AM.

2. The ratio (340 nm/380 nm) of steady-state $[Ca^{2+}]_i$ in resting osteoblasts kept in Hepes-buffered medium was $0{\cdot}82 \pm 0{\cdot}04$. A hyperosmotic stimulus (200 mosmol l^{-1} sucrose) produced a $[Ca^{2+}]_i$ transient with a peak ratio of $1{\cdot}28 \pm 0{\cdot}09$, which decayed with an apparent half-life ($t_{½}$) of $42{\cdot}7 \pm 2{\cdot}6$ s.

3. The hyperosmotically induced $[Ca^{2+}]_i$ transients were insensitive to verapamil, diltiazem or nifedipine, which excludes the involvement of dihydropyridine-sensitive Ca^{2+} channels in the process. Non-specific Ca^{2+} channel blockers (Mn^{2+}, Ni^{2+}, La^{3+} or Gd^{3+}) partially abolished the hyperosmotically induced $[Ca^{2+}]_i$ elevation, indicating the contribution of extracellular Ca^{2+} influx.

4. A hyperosmotic stimulus applied in Ca^{2+}-free medium (0·5 mM EGTA) lowered the $[Ca^{2+}]_i$ peak to a ratio of $0{\cdot}96 \pm 0{\cdot}08$ ($P < 0{\cdot}001$) compared with a Ca^{2+}-containing medium. This suggests that the $[Ca^{2+}]_i$ increase is due to extracellular influx, as well as release from an intracellular Ca^{2+} pool.

5. Application of thapsigargin (0·5 μM), a specific inhibitor of endoplasmic reticulum Ca^{2+}-ATPase, in Ca^{2+}-free medium caused transient $[Ca^{2+}]_i$ elevation to peak ratios of $1{\cdot}33 \pm 0{\cdot}09$, and completely abolished the $[Ca^{2+}]_i$ response to a hyperosmotic stimulus. This implies the existence of a thapsigargin-sensitive intracellular pool of Ca^{2+} that is mobilized by hyperosmotic stimulus.

6. Complete inhibition of the hyperosmotic Ca^{2+}-induced changes was obtained following exposure of cells to dihydrocytochalasin B (5 μM), whereas cell pretreatment with colchicine (5 μM) did not affect $[Ca^{2+}]_i$ increase.

7. The results suggest that a hyperosmotic stimulus generates a $[Ca^{2+}]_i$ transient through the mobilization of Ca^{2+} from an intracellular thapsigargin-sensitive pool and via bivalent and trivalent cation-sensitive Ca^{2+} influx. The integrity of microfilaments is essential for this $[Ca^{2+}]_i$ response.

Bone tissue is exposed to continuous mechanical pressure which leads to incessant remodelling (Wolff, 1892; Clinton & Lanyon, 1985). The applied external force is transmitted through the extracellular matrix to the cell membrane and cortical skeleton (Wang, Butler & Ingber, 1993). The mechanical stimulus is transduced into a sequence of biochemical processes involving elevation of Ca^{2+}, IP_3, prostaglandin E_2 or cAMP concentrations, followed by a late onset of cell proliferation (Sandy, Meghji, Farndale & Meikle, 1989; Reich & Frangos, 1991; Jones, Nolte, Scholübbers, Turner & Velter, 1991; Lanyon, 1993). Mechanical modulation of cytosolic calcium ($[Ca^{2+}]_i$) was also shown to occur in epithelial and endothelial cells (Enomoto, Furuya, Yamagishi & Maeno, 1992; Sigurdson, Sachs & Diamond, 1993).

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Exposure of cells to osmotic stress involves mechanical effects on the cell membrane and cytoskeleton. Hyposmotic stimuli were found to elevate $[Ca^{2+}]_i$ in osteosarcoma-derived bone cells (Yamaguchi, Green, Kleeman & Muallem, 1989). A hyperosmotic stimulus alters the intracellular pH (pH_i) in rat osteoblasts (Dascalu, Nevo & Korenstein, 1992) and causes an increase in $[Ca^{2+}]_i$ in lymphocytes (Grinstein, Dupre & Rothstein, 1982). In addition, the mechanisms responsible for pH_i regulation in an osteosarcoma cell line were modulated by $[Ca^{2+}]_i$ (Green & Kleeman, 1992), reflecting a complex interaction between pH_i and $[Ca^{2+}]_i$ (Dickens, Gillespie, Greenwell & Hutchinson, 1990). Therefore, a link might exist between hyperosmotic stimuli, pH_i and $[Ca^{2+}]_i$. Here we present evidence for $[Ca^{2+}]_i$ involvement in the response of osteoblasts to a hyperosmotic stimulus. The mechanism responsible for $[Ca^{2+}]_i$ changes is investigated and the role of cytoskeletal components in the response is assessed.

METHODS

Cell culture

An established cell line of rat calvaria, RCJ 1.20 (Aubin, Heersche, Merrilees & Sodek, 1982), generously donated by Dr J. E. Aubin (University of Toronto, Canada), was cultured in Eagle's minimum essential medium (MEM) (Biol. Industries, Beit-Haemek, Israel) containing 7·5% fetal calf serum (FCS), 4·5 mg ml^{-1} glutamine, 50 units ml^{-1} penicillin and 200 μg ml^{-1} streptomycin. The cells used in the experiments were seeded at an initial density of 1×10^5 cells cm^{-2} and kept at 37 °C in 5% CO_2 in air. Three to four days after plating, cells were collected from confluent cultures by 5–10 min trypsinization (0·05% trypsin and 0·02% EDTA in MEM). The proteolytic digestion was stopped by 2% FCS in MEM, the cells were briefly (1–2 s) sedimented by centrifugation at 7000 g and resuspended in MEM containing 0·2% FCS.

Ratiometric $[Ca^{2+}]_i$ imaging

Prior to plating and dye loading, cells were incubated at 37 °C for 30 min. Cells in suspension were plated on 22 mm diameter coverslips (No. 1, Superior, Germany) precoated with 3 μg ml^{-1} fibronectin, and left to attach to the surface for 20 min. Coating of coverslips with fibronectin did not interfere with $[Ca^{2+}]_i$ measurements, since, in a previous study (Savarese, Russell, Fatatis & Liotta, 1992), concentrations tenfold higher than those applied to the coverslips in the present study had no effect on $[Ca^{2+}]_i$ in RCJ or other cell types. After mild washing with MEM to remove unattached cells, osteoblasts were loaded for 20–25 min at 37 °C with 3 μM fura-2 AM. Subsequently, cells were washed twice and kept in a Hepes-buffered medium (see Solutions and materials) for 30 min prior to measurements, in order to minimize subcellular compartmentalization. Inspection of fura-2-loaded cells revealed no punctate distribution of the fluorescent dye and the autofluorescence of unloaded cells was negligible.

$[Ca^{2+}]_i$ analysis was performed using an image analysis system (TARDIS version 7.3; Applied Imaging, Hylton Park, Sunderland, UK). Coverslips with attached osteoblasts were placed on the stage of an inverted microscope (Nikon Diaphot) used in the epifluorescence mode (×40 oil-immersion objective lens) and equipped with a xenon lamp. $[Ca^{2+}]_i$ was assayed by ratio imaging of fura-2 AM (excitation, 340 and 380 nm; emission, 515 nm). A temperature-controlled perfusion chamber (Applied Imaging) was used to keep cells at a temperature of 32 °C. All media were maintained at 32 °C in a thermostatically controlled bath. Single cell images (10–40 cells per field) were measured by an intensified video camera (Photonic Science, Robertsbridge, UK). The video signal was averaged over four frames and averaged image pairs (340 and 380 nm) were captured every 5–15 s. Images were digitized at 256 × 256 pixels and ratios were calculated after background subtraction.

Intracellular Ca^{2+} calibration. Fura-2 AM signals were calibrated by addition of 5 μM ionomycin to cells kept in a 2 mM Ca^{2+} medium to obtain maximum fluorescence followed by addition of 10 mM EGTA with pH adjusted to >8·5 , in order to obtain the minimal fluorescence. Data are given as ratios of fluorescence at 340 nm/380 nm excitation and the $[Ca^{2+}]_i$ was calculated as previously described (Grynkiewicz, Poenie & Tsien, 1985) employing a Ca^{2+}–fura-2 dissociation constant of 224 nM.

Solutions and materials

All media were nominally HCO_3^- free. Hepes-buffered medium contained (mM): 145 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 Hepes and 0·5 mg ml^{-1} bovine serum albumin (BSA); adjusted to pH 7·3. Choline chloride and KCl media were prepared by isosmotic replacement of NaCl with choline chloride and KCl. Ca^{2+}-free medium was prepared by omitting $CaCl_2$ and including 0·5 mM EGTA. The osmolarity of isosmotic media was measured, using a vapour pressure osmometer (Wescor 5500, Logan, UT, USA), to be 300 ± 5 mosmol l^{-1}. The hyperosmotic stimulus was produced by addition of sucrose at a final osmolarity of 200 mosmol l^{-1} (the overall osmolarity was 500 mosmol l^{-1}). Preincubation periods for the different agents varied from 3–5 min for EGTA, to 30 min for dihydrocytochalasin B and colchicine.

This method of applying a hyperosmotic stimulus was chosen, since sucrose shows low absorbance in the excitation range. However, an artifact, related to viscosity effects, was observed during $[Ca^{2+}]_i$ measurement at much higher (ninefold) sucrose concentrations than employed in this study (Poenie, 1990; Busa, 1992), therefore we preferred not to apply any correction to our data. Fura-2 AM was purchased from Molecular Probes, Inc., Eugene, OR, USA. In a previous study of hyperosmotic effects (Dascalu, Nevo & Korenstein, 1992), defective fura-2 from a different supplier was used which did not respond to a hyperosmotic stimulus. Ionomycin, EGTA, BSA, colchicine, dihydrocytochalasin B, ryanodine, $MgCl_2$, $NiCl_2$, $LaCl_3$, $GdCl_3$ nifedipine, diltiazem and verapamil were obtained from Sigma Chemical Co., St Louis, MO, USA. The results are presented either as means ± S.E.M. of the indicated number of cells (n) analysed, or as representative tracings of individual experiments. Each experiment was repeated at least three times and comparisons were made using the Mann–Whitney U test.

RESULTS

Hyperosmotic stress induces a $[Ca^{2+}]_i$ transient

When measured by fura-2 AM fluorescence, the mean basal fluorescence ratio in RCJ cells ($n = 34$) was 0·82 ± 0·04 ($[Ca^{2+}]_i$, 76·2 ± 3·9 nM). Osteoblasts challenged

Table 1. Response of $[Ca^{2+}]_i$ to hyperosmotic stress of cells kept in Hepes-buffered medium compared with Na^+-free medium

	Peak fluorescence ratio	Time to peak (s)	Apparent $t_{½}$ of decay (s)
Hepes-buffered medium	1·28 ± 0·07 (42)	48·3 ± 3·1 (32)	42·7 ± 2·6 (32)
Na^+-free medium	1·17 ± 0·31 (43)	147·7 ± 12·1 (27)	166·4 ± 16·0 (24)
P	n.s.	< 0·001	< 0·001

Values are given as means ± S.E.M. of single cell recordings in at least four independent experiments, *n* is given in parentheses. Statistical significance was calculated using the Mann–Whitney test.

with a 200 mosmol l^{-1} hyperosmotic stimulus responded with a $[Ca^{2+}]_i$ transient ratio of 1·28 ± 0·09 ($n = 42$). A representative response from a single cell is shown in the left trace of Fig. 1. The elevated $[Ca^{2+}]_i$ decayed with an apparent half-life ($t_{½}$) of 42·7 ± 2·6 s ($n = 32$). Replacement of external sodium with choline (Fig. 1, right trace) increased both the rise time to peak and the apparent $t_{½}$ of decay of the response three- and fourfold, respectively (Table 1). This implies the involvement of external sodium in the process of $[Ca^{2+}]_i$ elevation and decay.

The effects of Ca^{2+} channel blockers and removal of extracellular Ca^{2+}

The contribution of extracellular Ca^{2+} influx to a hyperosmotic stimulus was studied. Inhibitors of voltage-gated Ca^{2+} channels, verapamil, diltiazem or nifedipine (10, 2 and 10 μM, respectively) did not influence the $[Ca^{2+}]_i$ elevation. Alternatively, exposure of cells to a depolarizing KCl medium (55 mM) or ryanodine (20 μM) did not affect the basal $[Ca^{2+}]_i$ levels or response to a hyperosmotic stimulus, and addition of valinomycin (2 μM) caused complete inhibition of the $[Ca^{2+}]_i$ elevation (data not shown). These experiments indicate the lack of involvement of membrane depolarization and voltage-gated channels in the $[Ca^{2+}]_i$ response to the hyperosmotic stimulus, and the abolishing effect of hyperpolarization of the cell membrane on $[Ca^{2+}]_i$ elevation. Mn^{2+} (0·5 mM), Ni^{3+} (50 μM), La^{3+} (100 μM) or Gd^{3+} (100 μM) significantly diminished hyperosmotically induced $[Ca^{2+}]_i$ elevation (Fig. 2*A* and *B*; data not shown for Mn^{2+} and Ni^{3+}), providing evidence for extracellular Ca^{2+} influx as a major source of the response.

When 0·5 mM EGTA was added to Ca^{2+}-free medium, the basal $[Ca^{2+}]_i$ decreased to a ratio of 0·76 ± 0·04 ($n = 42$) and the $[Ca^{2+}]_i$ transient induced by the hyperosmotic stimulus was markedly diminished (Fig. 3). Under these conditions the Ca^{2+} ratio transient was 0·96 ± 0·08 ($n = 25$), a value markedly lower than that observed in

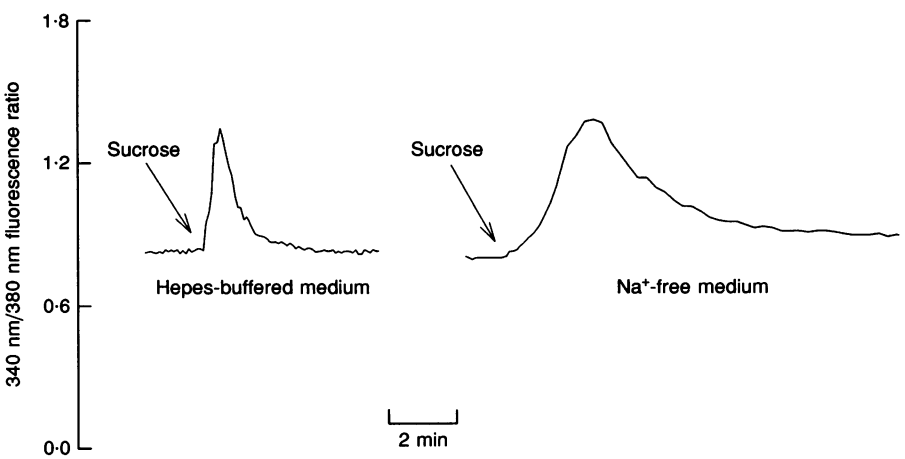

Figure 1. Effect of a hyperosmotic stimulus of sucrose (200 mosmol l^{-1}) on $[Ca^{2+}]_i$ in osteoblasts kept in Hepes-buffered or Na^+-free medium

Cytosolic Ca^{2+} was monitored by fura-2 AM employing an image analysis system (see Methods). Hepes-buffered medium (left trace) and Na^+-free medium (sodium replaced with an equimolar quantity of choline, right trace). Sucrose was added where indicated from a 1·6 M stock solution. Results are representative of at least four other independent experiments.

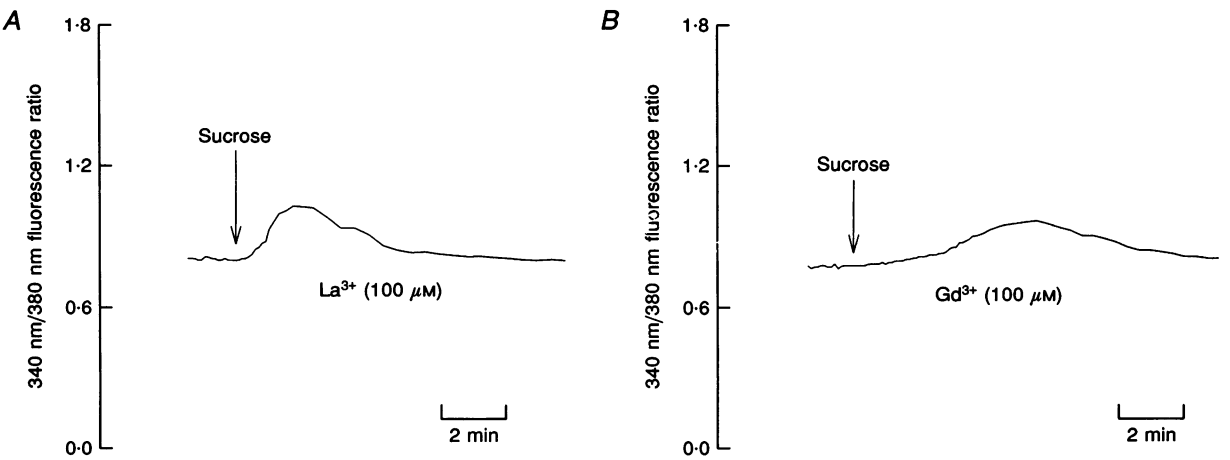

Figure 2. Inhibition of the $[Ca^{2+}]_i$ response to a hyperosmotic stimulus in cells exposed to La^{3+} (100 μM, *A*) and Gd^{3+} (100 μM, *B*)

Cells kept in Hepes-buffered medium were exposed for 3 min to the inhibitor in an isosmotic medium and the hyperosmotic load was added to the medium. Results are representative of at least three other independent experiments.

Ca^{2+}-containing medium ($P < 0{\cdot}001$). Subsequent challenge with 4 mM Ca^{2+} resulted in a rapid increase of $[Ca^{2+}]_i$ (Fig. 3). These experiments suggest that a hyperosmotically induced $[Ca^{2+}]_i$ transient resulted both from the mobilization of cellular Ca^{2+} as well as Ca^{2+} influx.

The role of intracellular Ca^{2+} stores

Thapsigargin, a specific inhibitor of endoplasmic reticulum Ca^{2+}-ATPase (Thastrup, Cullen, Droback, Hanley & Dawson, 1990), was used in order to evaluate directly the contribution of intracellular stores to a hyperosmotic stimulus response. Addition of 0·5 μM thapsigargin to cells resulted in a transient elevation of the fluorescence ratio ($1{\cdot}31 \pm 0{\cdot}07$; $n = 41$) and an apparent $t_{½}$ of $36{\cdot}2 \pm 1{\cdot}7$ s ($n = 36$). After the transient, $[Ca^{2+}]_i$ stabilized at a higher level than before the addition of thapsigargin (Fig. 4, left trace). Application of sucrose prior to the addition of thapsigargin (Fig. 4, right trace) reduced the response of

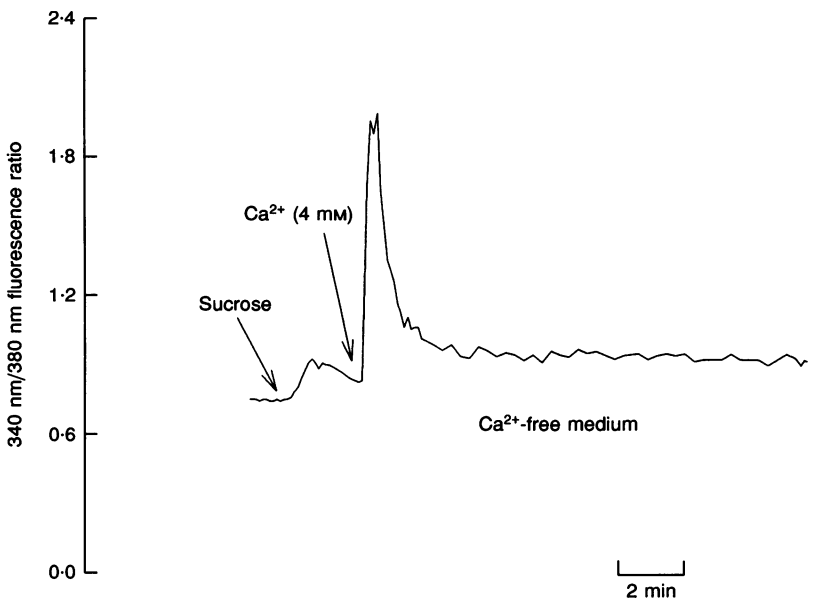

Figure 3. Effects of hyperosmotic stress applied in Ca^{2+}-free medium containing 0·5 mM EGTA

Cells were kept in Ca^{2+}-free medium for up to 5 min. A hyperosmotic stimulus was applied and 4 mM Ca^{2+} added while monitoring $[Ca^{2+}]_i$. Results are representative of at least four other independent experiments.

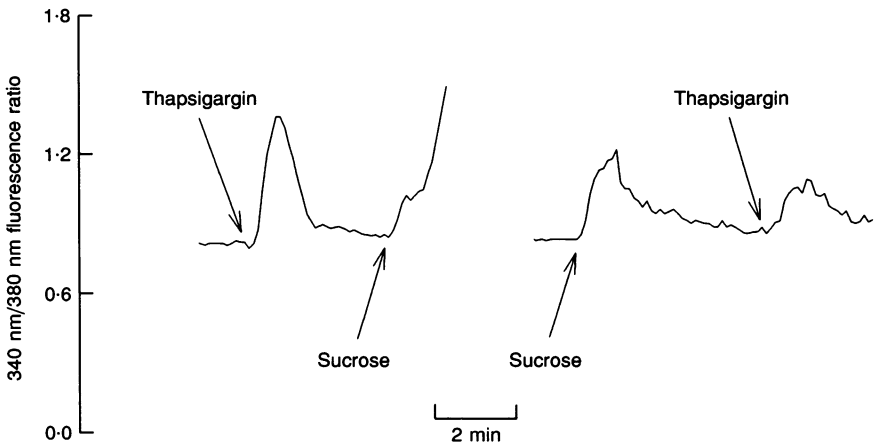

Figure 4. Non-additive effects of sucrose (200 mosmol l^{-1}) and thapsigargin (0·5 μM) on osteoblasts kept in Hepes-buffered medium

Left trace, sucrose was applied prior to thapsigargin. Right trace, thapsigargin was applied prior to sucrose. Results are representative of at least four other independent experiments.

the osteoblasts to the latter effector to a ratio of 1·0 ± 0·19 ($n = 12$; $P < 0·001$ compared with the thapsigargin response). A hyperosmotic stimulus applied after the thapsigargin treatment produced a dramatic $[Ca^{2+}]_i$ increase, which failed to return to baseline values (Fig. 4, left trace).

When cells were maintained in Ca^{2+}-free medium (0·5 mM EGTA), thapsigargin induced transient fluorescence ratio elevations of 1·33 ± 0·09 ($n = 31$) and an apparent $t_{½}$ of 19·8 ± 1·1 s ($n = 23$; $P < 0·001$ compared with the decay in Hepes-buffered medium). Challenge with thapsigargin prior to a hyperosmotic stimulus in a Ca^{2+}-free medium completely abolished the $[Ca^{2+}]_i$ transient (Fig. 5). In conclusion, these experiments suggest the existence of a limited thapsigargin-sensitive intracellular pool of Ca^{2+} that can be mobilized by a hyperosmotic stimulus.

The involvement of cytoskeleton in the hyperosmotic response

Colchicine (a tubulin array inhibitor) and dihydrocytochalasin B (a microfilament disrupter) were used in order to assess the involvement of cytoskeletal elements in the hyperosmotically induced $[Ca^{2+}]_i$ response. While colchicine (5 μM) did not prevent the hyperosmotically induced $[Ca^{2+}]_i$ transient (Fig. 6, left trace), preincubation of cells with dihydrocytochalasin B (5 μM) resulted in complete inhibition of the hyperosmotic effect (Fig. 6, right trace). These results suggest that actin-containing cytoskeletal elements may be involved in the $[Ca^{2+}]_i$ response to a hyperosmotic stimulus.

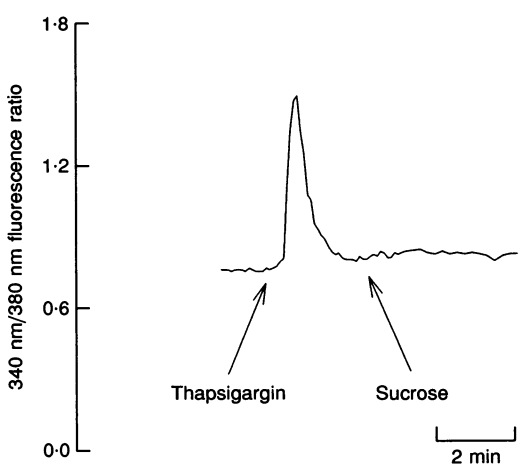

Figure 5. Effects of thapsigargin and osmotic stimulus applied to cells kept in Ca^{2+}-free medium

Thapsigargin (0·5 μM) was added prior to sucrose (200 mosmol l^{-1}). Results are representative of at least four other independent experiments.

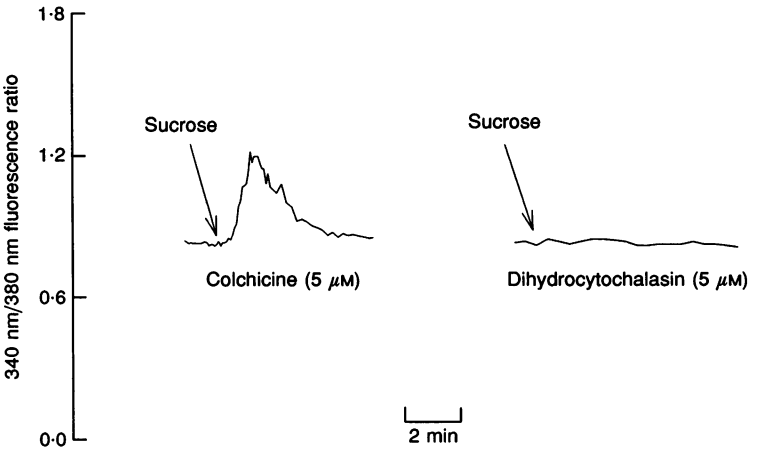

Figure 6. Influence of the cytoskeleton disrupting agents colchicine (5 μM, left trace) and dihydrocytochalasin B (5 μM, right trace)

Prior to hyperosmotic exposure, cells were preincubated for 30 min with the inhibitors. Results are representative of at least four other independent experiments.

DISCUSSION

The data presented in this study demonstrate that in RCJ osteoblasts a hyperosmotic stimulus causes transient elevation of $[Ca^{2+}]_i$. Similar to the mechanism of signal transduction following the activation of a large number of cell membrane receptors, the hyperosmotically induced elevation of $[Ca^{2+}]_i$ reflects both the mobilization of Ca^{2+} from thapsigargin-sensitive cellular stores and the influx of extracellular Ca^{2+} via a voltage-insensitive pathway. Microfilaments, central components of the cytoskeleton, appear to be involved in the transduction of this signal, since pretreatment with dihydrocytochalasin B abolished the hyperosmotic stimulus response.

A hyperosmotic stimulus triggers $[Ca^{2+}]_i$ influx into the cell

Several experimental approaches strongly suggest that the hyperosmotically induced response reflects an influx of extracellular Ca^{2+}. The responses obtained in Ca^{2+}-containing medium were significantly larger than in Ca^{2+}-free medium (Fig. 3). When the hyperosmotic stimulus was applied in Ca^{2+}-free medium, addition of Ca^{2+} caused an additional large $[Ca^{2+}]_i$ transient (Fig. 3). The inclusion of trivalent metal cations, like La^{3+} or Gd^{3+}, significantly attenuated the hyperosmotically induced $[Ca^{2+}]_i$ transient (Fig. 2*A* and *B*). These agents have been shown to inhibit Ca^{2+} influx through channels in a large number of experimental models (Tsunoda, 1993).

The hyperosmotically induced Ca^{2+} influx most probably proceeds via the 'capacitative' mechanism (Putney, 1990), since antagonists of voltage-sensitive Ca^{2+} channels had no effect on this response. Moreover, depolarization induced by high external K^+ caused no elevation of $[Ca^{2+}]_i$ and did not affect the hyperosmotically induced transient, whereas hyperpolarization caused by valinomycin completely inhibited the hyperosmotic response, implying a hyperpolarization-sensitive step in Ca^{2+} influx. As performing the hyperosmotic stimulus in the presence of extracellular EGTA caused a reduction in the $[Ca^{2+}]_i$ response and Ca^{2+} channel blockers did not affect the hyperosmotic response, it may be speculated that Ca^{2+} entry induced by a hyperosmotic stimulus is analogous to the yet uncharacterized receptor-activated Ca^{2+} channel. The possible involvement of ryanodine-sensitive receptors in the hyperosmotic stimulus response is unlikely, since ryanodine had no discernible effect on the response to a hyperosmotic stimulus.

A hyperosmotic stimulus mobilizes $[Ca^{2+}]_i$ from thapsigargin-sensitive stores

Two types of experiments support our claim that a hyperosmotic stimulus also causes mobilization of Ca^{2+} from cellular stores. A hyperosmotic stimulus induced a $[Ca^{2+}]_i$ transient in the absence of extracellular Ca^{2+}, albeit to a more limited extent than in its presence. Additionally, pretreatment of RCJ cells with thapsigargin in Ca^{2+}-free medium, resulted in depletion of Ca^{2+} from endoplasmic reticulum pools and completely abolished the hyperosmotic stimulus response (Fig. 5).

Our data suggest that a hyperosmotic stimulus mobilizes Ca^{2+} from thapsigargin-sensitive stores. Thapsigargin treatment of cells caused a distinct prolonged transient in Hepes-buffered medium compared with Ca^{2+}-free medium (left trace of Fig. 4 and Fig. 5). The extended transient

obtained in thapsigargin-treated cells kept in Hepes-buffered medium is compatible with refilling of the intracellular stores by extracellular Ca^{2+} influx, following exposure to thapsigargin, as previously shown in other cell types (Tsunoda, 1993). This supports the existence of capacitative Ca^{2+} influx in RCJ cells. It appears that the size of the hyperosmotic stimulus-sensitive pool is significant, since a hyperosmotic stimulus challenge prior to thapsigargin exposure resulted in a major decrease of the thapsigargin-induced $[Ca^{2+}]_i$ transient (see Fig. 4, right trace). It is difficult to interpret the results of the experiment illustrated in the left trace of Fig. 4. Despite earlier exposure to thapsigargin, there was potentiation of the response to a hyperosmotic stimulus and the elevated $[Ca^{2+}]_i$ did not return to baseline values within the time of the experiment. This phenomenon could be attributed to the influx of Ca^{2+} which was not offset by the sequestration of the elevated $[Ca^{2+}]_i$ into thapsigargin-sensitive stores. It implies that the depletion of Ca^{2+} stores by 0·5 μM thapsigargin alone was not sufficient to induce Ca^{2+} influx. Only the combined depletion of thapsigargin followed by a hyperosmotic stimulus seemed to trigger massive influx of Ca^{2+}. This interpretation is strengthened by our finding that higher concentrations of thapsigargin (e.g. 1·2 μM, not shown) produced $[Ca^{2+}]_i$ elevation that was much more dramatic, which also failed to return to baseline values. In *Xenopus* oocytes a similar phenomenon was previously described, since only prolonged exposure to thapsigargin resulted in stores depletion that induced Ca^{2+} influx (Lupu-Meiri, Beit-Or, Christensen & Oron, 1993).

A major component of the recovery of the osteoblast from the hyperosmotically induced $[Ca^{2+}]_i$ transient appears to proceed via Na^+–Ca^{2+} exchange. This was manifested by the significantly longer decay in Na^+-free medium (Table 1). The slower rate of $[Ca^{2+}]_i$ elevation suggests an additional role for extracellular Na^+. It is possible that the depletion of Na^+ from the external medium, which was previously shown to cause intracellular acidification due to the inactivation of the sodium–proton exchanger (Dascalu *et al.* 1992), influences either the mobilization of cellular Ca^{2+}, Ca^{2+} influx, or both.

Actin participates in $[Ca^{2+}]_i$ regulation following the hyperosmotic stimulus

The results suggest that the cytoskeleton may be involved in the response to the hyperosmotic stimulus. Whereas disruption of microtubuli had no effect on the transient $[Ca^{2+}]_i$ response, dihydrocytochalasin B, a microfilament disrupter, completely abolished it (Fig. 6). It is possible that actin or actin-binding proteins are required for transducing the hyperosmotic stimulus to both the cell membrane and internal Ca^{2+}-storing organelles. This complies with a general concept which relates mechanical changes initiated by Ca^{2+} to microfilaments rather than to microtubuli (Janmey, 1994). It is known that cell motility changes are generally controlled by $[Ca^{2+}]_i$, which can activate or reverse actin polymerization (Janmey, 1994). Actin was reported to be involved in events that are located downstream in the Ca^{2+} mobilization transduction pathway, as opposed to our findings of actin functioning upstream of the hyperosmotic stimulus response, since actin microfilament disruption annihilated the $[Ca^{2+}]_i$ transient.

Osmotic stimuli and the $[Ca^{2+}]_i$ response

Only a few reports demonstrate $[Ca^{2+}]_i$ transients in response to a hyperosmotic stimulus, all of them in cardiac cells (Allen & Smith, 1987). There are numerous reports which indicate that a hyperosmotic stimulus inhibits the induction of $[Ca^{2+}]_i$ transients by other stimuli (Parker & Zhu, 1987; Kazilek, Merkle & Chandler, 1988; Loechner, Knox, Connor & Kaczmarek, 1992). Hence this report extends previous findings to cells of non-cardiac origin. It is possible that the hyperosmotically induced $[Ca^{2+}]_i$ response in myocardiocytes and osteoblasts may be representative of a general pathway that couples membrane deformation to the $[Ca^{2+}]_i$ cascade. Indeed, the hyperosmotically induced Ca^{2+} transients were duplicated in avian chondrocytes (authors' unpublished results). Cell shrinkage following a hyperosmotic stimulus (increasing surface to volume ratio) leads to contraction of the cytoskeleton, as well as increased folding of the plasma membrane. This causes a higher local bending deformability of the membrane, as well as possible compression strain on the cytoskeleton. Direct mechanical perturbations, as well as hyposmotic effects, which have previously been reported to induce $[Ca^{2+}]_i$ elevation (e.g. in endothelium, see Sigurdson *et al.* 1993), which could be explained in terms of activation of stretch-sensitive channels. This possibility is unlikely in the case of a hyperosmotic stimulus, but cannot be fully excluded, especially in view of the inhibitory action of Gd^{3+}, a common inhibitor of stretch-activated channels.

A tempting speculation is that hyperosmotically induced $[Ca^{2+}]_i$ transients operate in tissues that are physiologically subjected to continuous mechanical stress. Hence, cardiac myocytes and osteoblasts may be representative of a class of tissues that display a specific, dedicated mechanism of mechanical stress transduction. Further studies of tissues that are physiologically subjected to mechanical pressure are necessary to validate this hypothesis.

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