# Disruption of Filamentous Actin Diminishes Hormonally Evoked $Ca^{2+}$ Responses in Rat Liver

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**Previous studies have suggested a role for the actin cytoskeleton in hormonally evoked $Ca^{2+}$ signaling in the liver. Here, we present evidence supporting a connection between filamentous actin (F-actin) organization and the ability of vasopressin and glucagon to increase cytosolic free-$Ca^{2+}$ ($[Ca^{2+}]_i$) levels. F-actin was disrupted in hepatic cells by perfusion of rat liver with cytochalasin D. Epifluorescence microscopy of subsequently isolated cells showed reduced cortical fluorescent phalloidin staining in cytochalasin D–treated liver cells. Cytochalasin D pretreatment of liver cells reduced the vasopressin-stimulated elevation of $[Ca^{2+}]_i$ by 60% and of glucagon by 50%. Experiments performed on cytochalasin D–treated cells using $Mn^{2+}$ as an indicator of $Ca^{2+}$ influx quenched fura-2 fluorescence signals following vasopressin administration. This indicates that a structurally intact cortical F-actin web is not a prerequisite for the influx of calcium. Therefore, the attenuation of the increase in cytosolic calcium observed in cytochalasin D–treated liver cells was likely caused either by the depletion of the calcium store by treatment with cytochalasin D or by the need for an intact cytoskeletal structure for its release. Because the resting level of calcium did not change in cells exposed to cytochalasin D, the latter is likely. The reduced $[Ca^{2+}]_i$ response may be the mechanism by which cytochalasin D pretreatment inhibits vasopressin-induced metabolic effects. Cytochalasin D pretreatment also decreased the ability of glucagon to stimulate gluconeogenesis and reduced the stimulation of $O_2$ uptake usually observed following glucagon administration. In conclusion, these results suggest that the hormonal elevation of $[Ca^{2+}]_i$ and resultant activation of specific metabolic pathways require normal F-actin organization.**


HORMONAL STIMULATION of the liver, as in other organs, involves in many cases, as a first step, the binding of the hormone to a specific receptor residing on the cell membrane outer surface and subsequent transfer of the signal to the cytosolic side of the plasma membrane. In many cases, a change in $Ca^{2+}$ flux follows. These fluxes are characterized by the influx of $Ca^{2+}$ and the release of $Ca^{2+}$ from intracellular storage pools. The result is an increase in cytosolic free-$Ca^{2+}$ ($[Ca^{2+}]_i$) levels. The elucidation of the mechanism by which hormones affect $Ca^{2+}$ distribution is a focal question of signal transduction, and is the subject of this study. The possibility that the cytoskeleton may play a role in the hormonal effects on $Ca^{2+}$ fluxes is examined here.

A major molecular constituent at the cytosolic side of the plasma membrane is actin, a component of the cytoskeleton; its concentration is approximately 1 mmol/L.[1-5] Visualization of the inner surface of the liver cell plasma membrane by dry-cleaving has revealed a uniform, filamentous web attached to the entire cytoplasmic side of the membrane.[6] Prior studies using the heavy-meromyosin–binding technique have identified the actin filaments within the entire cell periphery. They are especially conspicuous where the plasma membrane faces the Disse space or the bile canaliculi. The microfilaments are connected with each other, the plasma membrane, and intracellular organelles.[7] The observation that long-term administration of phalloidin to rats dramatically increased the microfilaments in hepatocytes heralded a new era in the detection of filamentous actin (F-actin), namely the use of fluorescent phallotoxins. Using fluorescent phalloidin staining, microfilaments were identified in the liver cell periphery, along the plasma membrane, and around the bile canaliculi.[8,9]

The notion that signal transduction by hormones might involve the cytoskeleton is based both on the abundance and strategic localization of F-actin and on observations showing that filament-disrupting agents, such as the cytochalasins, alter cellular responses.[10-12] Especially actin filaments were shown to influence the activity of ion channels.[13] Pretreatment of perfused livers with cytochalasin D in a previous study in our laboratory resulted in inhibition of the metabolic effects of vasopressin in the liver, such as the activation of glycogen phosphorylase.[14] These data indicated that F-actin on the cytoplasmic side of the plasma membrane might be involved in the chain of events connecting the binding of vasopressin to the receptor and in the final expression of its action, namely the increase in hepatic glucose output. Because the effects of vasopressin in the liver include an increase in $[Ca^{2+}]_i$, and glycogen phosphorylase is activated by increases in $[Ca^{2+}]_i$,[15,16] the possibility that cytochalasin D prevents the activation of glycogen phosphorylase because it interferes with the vasopressin-stimulated increases in $[Ca^{2+}]_i$ was examined in the present study. Because it was reported that signal transduction in the liver induced by glucagon might also involve the cytoskeleton, the influence of cytochalasin D on the effects of glucagon on $[Ca^{2+}]_i$ and gluconeogenesis was also explored.[17]

## MATERIALS AND METHODS

### *Animals*

Male rats (Harlan-Sprague-Dawley, Indianapolis, IN), either fed or fasted as indicated, weighing 130 to 200 g were used in all experiments.

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### *Materials*

[Arg$^8$]-vasopressin, $N^6$, 2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (cAMP), cytochalasin D, and albumin (bovine fraction V) were obtained from Sigma Chemical (St. Louis, MO). Collagenase A was purchased from Boehringer Mannheim (Indianapolis, IN). Glucagon was obtained from Eli Lilly & Co (Trenton, NJ). Tetramethyl isothiocyanate (TRITC) phalloidin and fura-2 were from Molecular Probes (Eugene, OR).

### *TRITC-Phalloidin Staining and Epifluorescence Microscopy*

To examine the effects of cytochalasin D on hepatocyte structure, fasted rats were perfused with Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, containing 2% albumin in situ as described by Friedmann and Rasmussen,[18] either with 5 μmol/L cytochalasin D or without cytochalasin D in controls. Subsequently, isolated cells were prepared. Isolated hepatocytes were fixed in 4% paraformaldehyde for 5 minutes at room temperature, washed in normal saline, and then refrigerated overnight. The following day, samples were washed in normal saline four times. After the last wash, samples were extracted in 0.15% Triton X-100 in normal saline for 2 minutes and then washed again in normal saline four times. Following the last wash, soft pellets of hepatocytes were formed by pulse-centrifugation in a microcentrifuge. The supernatant was removed, and the samples were resuspended in TRITC-phalloidin in normal saline (final concentration, 20 U/mL). After washing in normal saline three times, the cells were pipetted onto microscope slides and examined using a microscope (Leica Laborlux, Deerfield, IL) equipped for epifluorescence with a rhodamine filter cube and a 35-mm camera. Photographs were made using Kodak (Eastman Kodak, Rochester, NY) T-Max 400 35-mm film at 10-second exposures and both 32× and 100× microscope objectives.

### *Measurement of Gluconeogenesis in the Perfused Liver*

Livers from overnight-fasted rats were perfused in situ at 32°C as described previously by Friedmann and Rasmussen.[18] In short, livers were perfused with regular KRB containing 2% bovine serum albumin for 60 minutes. In some experiments, 5 μmol/L cytochalasin D was also added as indicated. An aliquot of the perfusate was taken for glucose determination, and substrates were added to produce a final concentration of 10 mmol/L sodium lactate and 1 mmol/L sodium pyruvate. Glucagon and vasopressin were also added to the reservoir when indicated. After perfusing the liver for an additional 60 minutes, aliquots of the perfusate were taken for measurement of the glucose concentration. Differences in perfusate glucose content between 60 and 120 minutes were used to calculate the rate of gluconeogenesis.

### *Measurement of Oxygen Uptake*

Oxygen uptake in perfused liver was measured at 32°C as described previously.[19] Perfusate oxygen content was monitored continuously by a Clark-type oxygen electrode attached to a YSI model 53 oxygen monitor and a recorder (Yellow Springs Instruments, Yellow Springs, OH). $O_2$ uptake was calculated as the difference between influent and effluent $O_2$ content. Livers from fed rats were perfused in the regular KRB containing 10 mmol/L glucose with or without 5 μmol/L cytochalasin D. Glucagon was added to the perfusate after 60 minutes.

### *Preparation of Isolated Hepatocytes*

Hepatocytes were isolated using the collagenase digestion technique,[20] with some modifications. The liver was perfused in situ for 30 minutes via the portal vein using $Ca^{2+}$-free KRB containing 1 mmol/L EGTA and 10 mmol/L glucose with (test) or without (control) 5 μmol/L cytochalasin D. After this period, 0.05% (wt/vol) collagenase was added to the perfusion apparatus and the perfusion continued until the liver surface appeared soft. The liver was removed, placed in a petri dish containing regular KRB and collagenase, and minced at room temperature. The liver suspension was filtered through cotton gauze. After filtration, cells were washed first with nominally $Ca^{2+}$-free KRB and then twice with regular KRB, pelleted, and counted. Cell viability was assessed by trypan-blue exclusion.

### *Measurement of $[Ca^{2+}]_i$ With Fura-2 AM*

The $[Ca^{2+}]_i$ level was measured in isolated cell preparations as in previous experiments in our laboratory.[21,22] Hepatocytes prepared from control or cytochalasin D–treated livers, were loaded with fura-2 AM on a rocker at room temperature for 45 minutes under a constant supply of 95% $O_2$/5% $CO_2$. After this period, the cells were sedimented by centrifugation and resuspended in a buffer containing 1.25 mmol/L probenecid to block the export of fura-2 from the cells.[23] All subsequent solutions for cell suspensions contained probenecid (1.25 mmol/L). The fluorescence of fura-2 AM–loaded cells was monitored at 37°C in an LS-5 Perkin Elmer (Norwalk, CT) fluorescence spectrophotometer. The instrument was controlled by a personal computer with the help of a free program developed in-house by Perkin Elmer personnel (Dean Brown) specifically for instrument control in models LS-5 and LS-3B. The program excited the cells alternatively at 340 and 380 nm and recorded the emission at 510 nm. For measuring and correcting for autofluorescence, each cell preparation was evaluated before loading with the dye in conditions that were otherwise identical to those used for the fura-2–loaded cells. The value obtained for autofluorescence was subtracted from all subsequent values obtained after the cells were loaded with the dye, to obtain fluorescence data from fura-2 emission itself without the contribution of autofluorescence from the cells or other components in the cuvette. $[Ca^{2+}]_i$ values were calculated from the fluorescence ratio recordings at 340 and 380 nm according to the equation developed and described by Grynkiewicz et al.[24] Thus, $[Ca^{2+}]_i = K_d(R - R_{min}R_{max} - R)$ $(Sf_2/Sb_2)$, where R is the ratio $F_1/F_2$ at the two wavelengths, S is the proportionality coefficient, $f_2$ is free dye at $\lambda_2$, and $b_2$ is bound dye at $\lambda_2$. The $K_d$ was 224 nmol/L. $R_{max}$, $R_{min}$, and $Sf_2/Sb_2$ were determined at the end of each experiment by measuring the 510-nm emission at 340 and 380 nm excitation after sequential additions of Triton X-100 and EGTA to the cell suspensions. Because $R_{max}$ and $R_{min}$ were both measured in the same instrument, any wavelength biases influence them equally. The advantage of using ratios for the calculations instead of total fluorescence intensities was discussed in detail by Grynkiewicz et al.[24]

### *Measurement of $Mn^{2+}$ Influx*

Liver cells were loaded with fura-2 as already described. $Mn^{2+}$ influx (a putative marker in the liver for vasopressin-evoked $Ca^{2+}$ influx) was measured essentially as described by Kass et al.[25,26] The fluorescence signal at 510 nm was recorded at the excitation wavelength of 334 nm. The initial readings were amplified to a set point. The quenching of the fluorescence signal was taken as an indication of $Mn^{2+}$ influx. The initial rate of fluorescence decline is expressed in terms of arbitrary fluorescence units per minute.

### *Statistical Analysis*

The data were analyzed by the paired *t* test using a computer program (Primer Biostatistics, 1988; McGraw-Hill, New York, NY).

## RESULTS

The effectiveness of cytochalasin D treatment of the liver for microfilament organization was evaluated by electron microscopy and TRITC-phalloidin staining. Actin staining with TRITC-phalloidin showed pronounced differences (Fig 1). In control cells, a prominent actin band surrounding the entire periphery was found. In cells prepared from cytochalasin D–treated livers,

there was a pronounced change in cortical actin: the actin band was disrupted and became discontinuous. In addition, Fig 1 also demonstrates that cytochalasin D treatment of perfused livers results in long-term disruption of the actin structure, which is visible in subsequently isolated liver cells. Thus, during the experimental period, the effects of cytochalasin D were not reversed.

Next, the effect of cytochalasin D pretreatment on the vasopressin-induced changes in $[Ca^{2+}]_i$ was measured (Fig 2). Vasopressin administration was followed by a sharp transient increase of about 400 nmol/L in $[Ca^{2+}]_i$. In cytochalasin D–treated cells, the increase in $[Ca^{2+}]_i$ was less than 25% of that observed in the control. These experiments were repeated four to eight times. The mean value for baseline $[Ca^{2+}]_i$ was 455 nmol/L. Vasopressin increased $[Ca^{2+}]_i$ to 970 nmol/L (mean of four experiments). Cytochalasin D–treated cells showed a reduction to 635 nmol/L (mean of eight experiments). The basal value for $[Ca^{2+}]_i$ was not affected by cytochalasin D, and was 465 nmol/L. Thus, cytochalasin D treatment significantly reduced the increase of $[Ca^{2+}]_i$ usually observed following vasopressin administration.

Because cytochalasin D treatment does not interfere with the production of inositol trisphosphate ($IP_3$),[14,27] the decrease in the calcium response to vasopressin in cytochalasin D–treated liver must have occurred after the production of $IP_3$. Thus, it could be the result of a decrease in the influx or the release of $Ca^{2+}$ from intracellular storage sites. To evaluate whether cytochalasin D pretreatment interferes with the influx or the release of $Ca^{2+}$ from intracellular stores, the effect of the treatment on vasopressin-stimulated $Mn^{2+}$ entry was measured. It has been demonstrated that $Mn^{2+}$ enters the liver cell through the same voltage-independent, receptor-operated channel as $Ca^{2+}$ and therefore can be used as an indicator of $Ca^{2+}$ entry.[25,26,28,29] As described by Kass et al,[25] $Mn^{2+}$ competes with $Ca^{2+}$ for entry into cells via receptor-operated calcium channels. The measurement of $Mn^{2+}$ entry as an indicator of $Ca^{2+}$ entry is based on the ability of $Mn^{2+}$ to bind to fura-2 with a higher affinity than $Ca^{2+}$. This results in the quenching of the fluorescence signal. Thus, when $Mn^{2+}$ is present in the buffer and an agonist is added (for instance, vasopressin; Fig 3), the decrease in fluorescence indicates and is proportional to the stimulation of $Mn^{2+}$ influx through receptor-operated $Ca^{2+}$ channels. Because the cells in Fig 3 were prepared from cytochalasin D–treated livers, which resulted in the disruption of the actin network (Fig 1), one can conclude from the experiments presented in Fig 3 that vasopressin stimulation of $Mn^{2+}$ (or $Ca^{2+}$) influx does not require the presence of an intact cytoskeletal actin network and occurs even when the actin network is disrupted by cytochalasin D.

The effects of vasopressin on $[Ca^{2+}]_i$ and glucose release are mediated by increases in $IP_3$ levels,[30,31] while the effects of glucagon on $[Ca^{2+}]_i$ are likely mediated by increases in cAMP levels.[32] It was therefore of interest to examine whether cytochalasin D also affects the glucagon-evoked increases in $[Ca^{2+}]_i$ or whether its effect is specific for hormones that act by increasing $IP_3$ levels. In the present study, we found that the increases were considerably less than the increases observed following vasopressin administration. However, cytochalasin D treatment inhibited the increases (data not shown). In agreement with the report by Kass et al,[25] $Mn^{2+}$ quenching was not stimulated by glucagon. Because of the connection between the ability of glucagon and cAMP to affect ion distribution, especially $Ca^{2+}$ fluxes, and their stimulatory effect on glucose production, the effects of cytochalasin D pretreatment on glucose production and $O_2$ uptake were measured. The basal rate of $O_2$ uptake was 2.84 ± 0.32 μmol/min/g liver. Glucagon ($10^{-8}$ mol/L) stimulated $O_2$ uptake in the perfused liver by 28% ± 0.9% (mean of three experiments). Cytochalasin D pretreatment reduced the increase to 11% ± 1.5%. Glucagon stimulation of gluconeogenesis was completely blocked by cytochalasin D pretreatment (Fig 4).

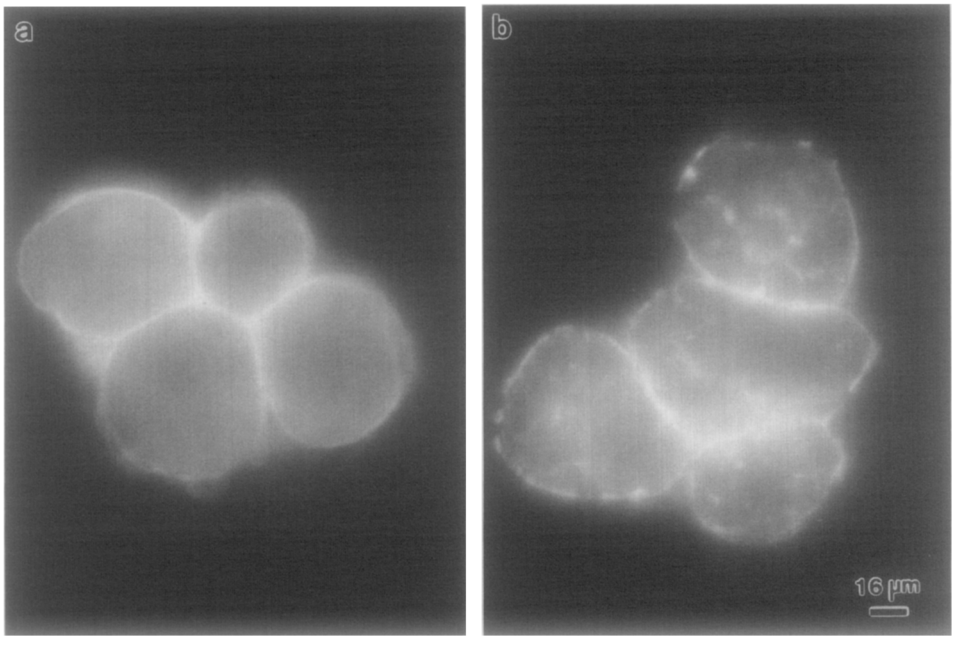
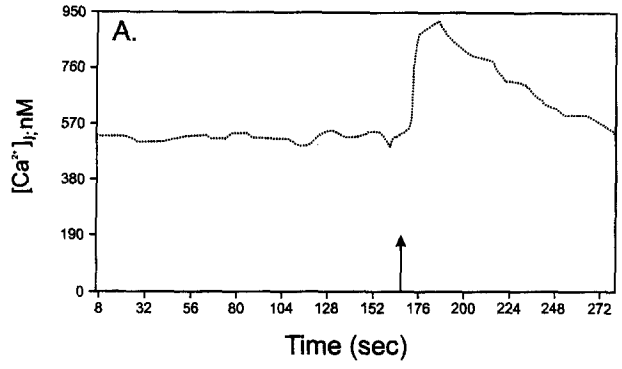



**Fig 1. Effects of cytochalasin D on actin structure. Representative micrographs of TRITC-phalloidin–stained hepatocytes prepared from livers perfused without (a) or with (b) 5 μmol/L cytochalasin D. Photographs were made with the 100× objective.**



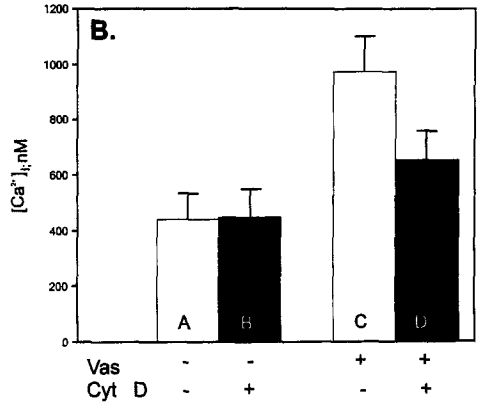




**Fig 2. Effects of cytochalasin D treatment on the vasopressin-evoked increase in $[Ca^{2+}]_i$. Cells were prepared from livers perfused either in the absence (control) or in the presence (experiment) of cytochalasin D and loaded with fura-2. (A) Representative recording of the increase in $[Ca^{2+}]_i$ observed after addition of 50 nmol/L vasopressin to a control cell preparation at the time indicated. (B) Results in control cells: (A), cells from livers perfused with cytochalasin D, (B) cells plus 50 nmol/L vasopressin, (C) cells from livers perfused with cytochalasin D and (D) exposed to 50 nmol/L vasopressin. Results are the mean ± SE of 4-8 experiments.**

## DISCUSSION

It is agreed that hormones acting through either $IP_3$ or cAMP affect $Ca^{2+}$ distribution in the liver. Both types of hormones stimulate the influx of $Ca^{2+}$ and the release of $Ca^{2+}$ from intracellular storage pools and increase $[Ca^{2+}]_i$.[33-38] However, despite the agreement on these observations, no consensus has been reached as to the mechanism by which hormones and second messengers elicit the redistribution of $Ca^{2+}$.

The major questions relate to the possible connection between the two processes, namely the influx of $Ca^{2+}$ and its release from the endoplasmic reticulum. That such a connection exists was shown by the use of thapsigargin, an inhibitor of the endoplasmic reticulum $Ca^{2+}$-ATPase.[31] Administration of this agent to the liver and other organs released stored $Ca^{2+}$ and stimulated $Ca^{2+}$ influx. This observation led to the concept of "capacitative $Ca^{2+}$ entry." This concept suggests that $Ca^{2+}$ release leads to $Ca^{2+}$ entry. Furthermore, the concept was applied to the mechanism for the action of $IP_3$, assuming that $Ca^{2+}$ depletion is a primary event, which leads to the subsequent influx of $Ca^{2+}$. The exact nature of the coupling process between the two events, $Ca^{2+}$ release and influx, is not clear and is being investigated.[39] Based on numerous studies in our laboratory and others, we proposed that cytoskeletal elements might serve as a link between the two compartments and the $Ca^{2+}$ influx and efflux processes.[40]

The results presented here support this suggestion. They show that disruption of the microfilamental structure by cytochalasin D interferes with the ability of both vasopressin and glucagon to increase $[Ca^{2+}]_i$. This indicates that the integrity of the cytoskeleton is a requirement for hormonal stimulation of $Ca^{2+}$ fluxes. However, these results do not distinguish between influx and efflux; either or both could be affected by disrupting the microfilaments. The results also do not resolve the question of whether the microfilamental structure itself or elements associated with it are involved in the $Ca^{2+}$ movement. Microinjection of $IP_3$ into isolated hepatocytes clearly showed that $IP_3$ evokes $Ca^{2+}$ entry both directly and indirectly, by the capacitative-entry process.[29,35]

To distinguish between influx and efflux processes, following vasopressin stimulation, we evaluated the ability of $Mn^{2+}$ to enter the liver cell through the same channel as $Ca^{2+}$.[25,26,29] Cytochalasin D pretreatment of the liver did not block vasopressin-stimulated $Mn^{2+}$ entry, as indicated by an increase in the

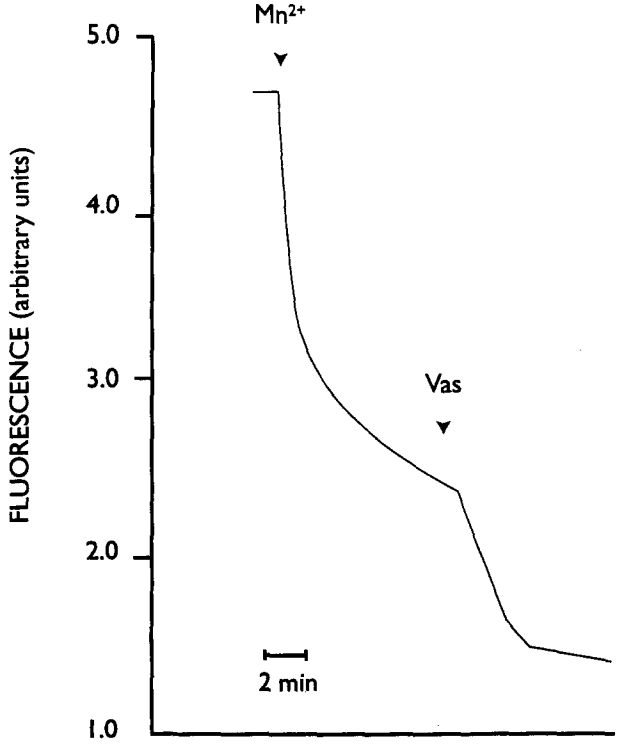



**Fig 3. Effects of cytochalasin D treatment on fura-2 fluorescence in the presence of $Mn^{2+}$. Cells were prepared from livers perfused with cytochalasin D and loaded with fura-2. Cells were excited at 334 nm, and emission at 510 nm was recorded. After reaching a stable baseline, 0.5 mmol/L $MnCl_2$ was added to the cuvette (arrow). The subsequent quenching of the fluorescence signal indicates $Mn^{2+}$ influx ($Mn^{2+}$ is a putative marker for $Ca^{2+}$ influx). Addition of 50 nmol/L vasopressin to the cuvette at the indicated time resulted in further quenching of the fluorescence signal, indicating additional $Mn^{2+}$ entry via $Ca^{2+}$ channels. Figure is representative of a set of 4 experiments with similar results.**

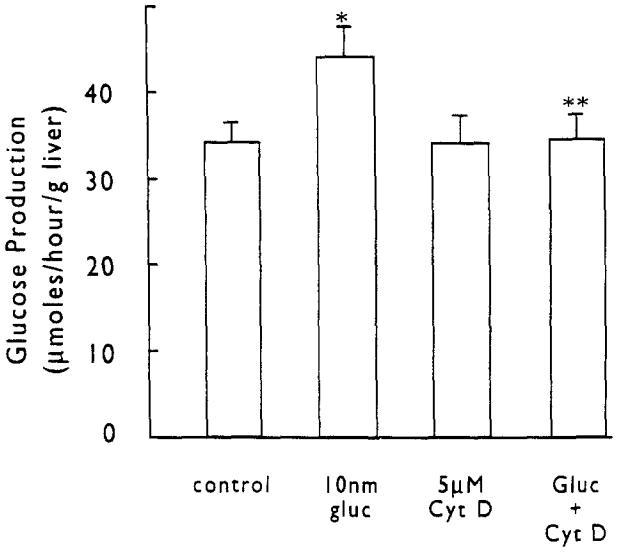



**Fig 4. Effects of cytochalasin D on the stimulatory effect of 10 nmol/L glucagon on gluconeogenesis in the perfused liver. *P = .003, control *v* glucagon. **P = .009, cytochalasin D *v* cytochalasin D plus glucagon.**

quenching of the fluorescent signal (Fig 3). This could be an indication that an intact filamentous web is not essential for opening the $Ca^{2+}$ influx channel. This observation is in line with reports that an $IP_3$-gated channel activity purified from the hepatic plasma membrane fraction exhibits channel activity when it is reconstructed into lipid bilayers.[41] A more likely explanation for the decrease in the response of $[Ca^{2+}]_i$ following disruption of the microfilaments seems to be that the microfilaments are involved in the $Ca^{2+}$ release process.

An additional possible reason for the decrease in the calcium response to hormonal stimulation could be the depletion of calcium stores by cytochalasin D treatment. However, the observation that resting calcium levels were the same in control and cytochalasin D–exposed liver cells argues against such an interpretation. Indeed, it was unexpected that no difference in $[Ca^{2+}]_i$ was found between cytochalasin D–treated and untreated livers (Fig 2). This could be interpreted as an indication of the involvement of microfilaments only in agonist-operated, not in resting, unstimulated calcium fluxes. Clearly, more studies are needed to clarify this question.

As in the liver, in a recent study, cytochalasin D treatment of NIH 3T3 cells was reported to inhibit agonist-stimulated calcium mobilization.[42] In that study, cytochalasin D treatment did not block the stimulatory effect of thapsigargin on the emptying of $Ca^{2+}$ stores and influx of $Ca^{2+}$. Thus, according to the data obtained both in the liver and in NIH 3T3 cells, an intact cytoskeleton is a prerequisite for agonist-induced calcium mobilization. However, the exact mechanism and the connection between the cytoskeleton and the increases in $[Ca^{2+}]_i$ remain to be elucidated.

Manganese could not be used to study glucagon-stimulated $Ca^{2+}$ influx, because the channel opened by glucagon is different from the vasopressin-stimulated channel and does not carry manganese.[25] The glucagon and cAMP–evoked influx of $Ca^{2+}$ precedes the efflux of $Ca^{2+}$,[37] and is likely due to the activation of a cyclic nucleotide–gated cation channel. This channel is similar in structure to the cyclic nucleotide–gated channels in sensory systems and was demonstrated to be present also in the liver.[43,43a] Therefore, although the experiments show a reduction of the $Ca^{2+}$ response to glucagon in cytochalasin D–treated livers and a blockade of the gluconeogenic response, a $Ca^{2+}$-dependent effect,[32] they do not indicate whether $Ca^{2+}$ influx, efflux, or both are affected. Further studies are needed to clarify this question.

The present data indicate a possible connection between the ability of hormones to increase $[Ca^{2+}]_i$ and the cytoskeleton. Disruption of cytoskeletal elements is associated with a loss of the effectiveness of both vasopressin and glucagon to alter $Ca^{2+}$ fluxes in the liver. Because similar results were also obtained in a different cell line, NIH 3T3 cells,[42] it may be that involvement of the cytoskeleton in agonist-evoked calcium fluxes is a widespread phenomenon. Indications that the receptors for $IP_3$ are associated with the cytoskeleton are abundant and have been discussed by Kraus-Friedmann[40] in detail. In addition to $IP_3$, diacylglycerol-stimulated increases in actin nucleation were also noted.[44] Also, actin cross-linking proteins were suggested to play a role as integrators of protein kinase C and $Ca^{2+}$/calmodulin signals. In many systems, connections between hormones acting by cAMP and cytoskeletal changes were also noted.[45] Indeed, studies on the effects of cAMP on cytoskeletal reorganization are too numerous for inclusion here; therefore, only a few randomly selected examples are presented. Protein kinases are activated by cAMP, and it was noted that the substrate specificity of these enzymes was achieved, at least in part, by the formation of supramolecular complexes between enzyme and subcellular structures containing their substrates, such as microtubule-associated proteins.[46] Secretion in parotid acinar cells evoked by isoproterenol or dibutyryl cAMP is associated with changes in fibrin and actin labeling.[47] Alterations in the labeling of F-actin and α-actinin in bladder carcinoma cells were described.[48] Vasopressin in the kidney—where it acts by increasing cAMP levels—was shown to depolymerize F-actin.[49] In endothelial cells grown in cell culture, 8-bromo cAMP was shown to affect the distribution of vimentin intermediate filaments and cytoskeletal-associated proteins, such as α-actinin and plectin, in addition to F-actin. Thus, the effects of cAMP may involve the cytoskeleton as a whole.[50]

It was reported that the effects of cAMP on the cytoskeleton are counteracted by insulin, similarly to the metabolic effects.[51] These observations and the present data demonstrating that the inhibition of both vasopressin- and glucagon-evoked calcium fluxes by cytochalasin D results in inhibition of the metabolic effects of these hormones reinforce the notion that the ion fluxes are an essential link in the chain of events leading to the metabolic effects.[32]

The studies presented herein indicate a possible role for the cytoskeleton in agonist-stimulated increases in cytosolic free-calcium levels. The elucidation of the mechanism by which the cytoskeleton interfaces with the changes produced during the process of signal transduction is necessary to further the understanding of signal transduction processes involving calcium fluxes.

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