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# Hormone-induced actin polymerization in rat hepatoma cells and human leucocytes

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Treatment of rat hepatoma cells with insulin, glucagon, thyroxine ($T_4$) and tri-iodothyronine ($T_3$) caused a concentration-dependent decrease in the monomeric actin content as measured by the deoxyribonuclease-I inhibition assay. Similarly, human peripheral blood neutrophils responded with a decrease in monomeric actin content when stimulated with $T_4$, $T_3$ and the adrenergic agonists phenylephrine and isoprenaline. The effect of phenylephrine could be blocked by phentolamine, demonstrating the specificity of the interaction. These observations suggest that hormone-induced actin changes might be an important event in response to both cell-surface-reactive hormones, such as insulin, glucagon and adrenergic agents, and those hormones that act through intracellular receptors, such as thyroid hormones. It is suggested that changes in actin state may have a role in metabolic regulation and cell growth.

Actin is a major component of the cytoskeleton and undergoes reversible alteration in its state during many cellular events. Changes in actin polymerization have been demonstrated in platelets after thrombin stimulation (Carlsson *et al.*, 1979; Pribluda *et al.*, 1981; Fox & Phillips, 1981), in neutrophils after chemotactic-peptide stimulation (Rao & Varani, 1982; Fechheimer & Zigmond, 1983; Howard & Meyer, 1984), and in lymphocytes after lectin stimulation (Rao, 1984). These changes are initiated in response to stimuli which act through cell-surface receptors and involve changes in cell morphology. Changes in cell morphology are also seen in response to peptide hormones and growth factors whose binding to cell-surface receptors is known to initiate specific cellular events. These include corticotropin (Ramachandran & Suyama, 1975; Cheitlin & Ramachandran, 1981), thyrotropin (Fayet *et al.*, 1971; Rapoport & Jones, 1978; Tramontano *et al.*, 1982; Avivi, 1982), follitropin (Tung *et al.*, 1975; Hutson, 1978; Lawrence *et al.*, 1979) and lutropin (Lawrence *et al.*, 1979) among peptide hormones, and epidermal growth factor (Gonzalez *et al.*, 1981) and nerve growth factor (Monaco *et al.*, 1977; Schubert *et al.*, 1978) among growth stimulators. Noradrenaline, a non-peptide hormone, also elicits a similar response (Balter *et al.*, 1977). These observations suggested to us that stimulation of cells with hormones might lead to demonstrable changes in actin polymerization. In the present study we demonstrate that changes in actin polymerization occur very rapidly in hormonally stimulated cells. Further, we discovered that the changes in actin polymerization occur not only with hormones that act through cell-surface receptors, but also with hormones that act through intracellular receptors, such as $T_4$ and $T_3$ (Oppenheimer, 1979).

Abbreviations used: $T_4$, thyroxine, $T_3$, tri-iodothyronine; DNAase, deoxyribonuclease.

## Materials and methods

### *Reagents*

DNA type I, ethidium (homidium) bromide, rabbit skeletal-muscle actin, $T_4$, $T_3$, phenylephrine hydrochloride and isoprenaline (isoproterenol) hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. DNAase I, bovine insulin and glucagon were obtained from Calbiochem–Behring Corp., La Jolla, CA, U.S.A. Phentolamine was given by Ciba–Geigy, Summit, NJ, U.S.A.

### *Cells*

*Rat hepatoma cells.* Continually growing ethionine-induced rat hepatoma cells (given by Dr. H.

Shinozuka, University of Pittsburgh) were maintained in Eagle's minimum essential medium supplemented with 10% (v/v) foetal-calf serum (Gibco, Grand Island, NY, U.S.A.), penicillin, streptomycin and kanamycin in a humidified incubator at 37°C in an atmosphere of air/$CO_2$ (19:1). Stock cultures were routinely subcultured (1:10) and grown in 75 $cm^2$ plastic flasks. On the day of the experiment, the cells were scraped with a rubber policeman, washed three times in Hanks' balanced salt solution and suspended in 5 mM-Tris/HCl/0.15 M-NaCl buffer, pH 7.4.

*Human polymorphonuclear leucocytes.* Fresh blood (30–40 ml) was obtained by venepuncture from volunteer donors (approved by the Biomedical Institutional Review Board, University of Pittsburgh), and defibrinated by the use of glass beads and constant rotation for 10–20 min. The defibrinated blood was subjected to the Ficoll/Hypaque density-gradient technique to remove mononuclear cells. The red-cell pellet was used to isolate polymorphonuclear leucocytes by a dextran sedimentation technique (Böyum, 1968). The red cells were removed by lysis (with 0.83% $NH_4Cl$/0.05% $KHCO_3$/0.1 mM-EDTA for 3 min). The cells were washed twice with Hanks' balanced salt solution and suspended in a physiological buffer with the following composition: 138 mM-NaCl, 4.5 mM-KCl, 1.2 mM-$P_i$, 1.2 mM-$MgSO_4$, 1.3 mM-$CaCl_2$, 5.6 mM-glucose, 5 mM-$NaHCO_3$ and 20 mM-Hepes, pH 7.4 at 37°C (buffer A).

*Cell treatment.* Cell suspension (100 $\mu$l; $3 \times 10^6$–$4 \times 10^6$ cells/ml) was incubated with various hormones (in 1 $\mu$l volume) for 5 min at 37°C. At the end of the incubation period, the cells were lysed with 100 $\mu$l of the lysis buffer (described below), and 1 min after lysis, 100 $\mu$l of the lysate was added to the assay mixture (described below). The effect of phentolamine on the phenylephrine-induced changes was studied by preincubating the cells for 5 min with phentolamine, followed by 5 min incubation with phenylephrine.

### *Measurement of monomeric actin (G-actin)*

Alterations in actin state were quantified by determining the concentration of monomeric actin by the DNAase-I inhibition assay (Blikstad *et al.*, 1978). The assay is based on the concentration-dependent inhibition of DNAase I activity by G-actin. The activity of the enzyme is monitored by the decrease in fluorescence intensity of an ethidium bromide–DNA complex, occurring as a result of DNA digestion produced by DNAase I (Laub *et al.*, 1981). The DNAase-I inhibitory activity of cell lysates is proportional to the amount of G-actin in the cells. Cell lysates were prepared by treating 100 $\mu$l of the cell suspension with 100 $\mu$l of buffer A containing 2% (v/v) Triton X-100 and 40 $\mu$g of DNAase I/ml (lysis buffer); 100 $\mu$l of the lysate was used in the assay. The assay mixture contained 60 $\mu$g of DNA/ml, 1.5 $\mu$M-ethidium bromide, 0.1 mM-$CaCl_2$ and 1 mM-$MgCl_2$ in 2.5 mM-Tris/HCl buffer (pH 8.0). The fluorescence emission of the mixture was adjusted to 90 relative fluorescence units (Perkin–Elmer LS-3 spectrofluorimeter, set at 520 nm excitation and 602 nm emission) and the temperature was maintained at 30°C. The DNAase I activity in the absence of actin was determined by the addition of 100 $\mu$l of the diluted lysis buffer (equivalent to 2 $\mu$g of the enzyme) to the assay mixture (2.5 ml) in the cuvette and monitoring the decrease in fluorescence over a 2 min period, as the DNA hydrolysis proceeded. Under these conditions both nuclear and cytoplasmic G-actin is measured. A calibration curve was constructed by using rabbit skeletal-muscle actin to inhibit the enzyme activity (Varani *et al.*, 1983). The amount of G-actin in the cell lysates was determined from the standard curve.

### *Measurement of total protein*

Protein content of the cell lysates was determined by the dye-binding method (Bradford, 1976), with bovine $\gamma$-globulin as the standard, made up in buffer A containing 2% (v/v) Triton X-100. The actin values were expressed as $\mu$g of G-actin/100 $\mu$g of total protein in the sample.

## Results

### *Effect of insulin and glucagon on the actin state in rat hepatoma cells*

Fig. 1(*a*) shows the effect of insulin on the G-actin content in rat hepatoma cells as measured by the DNAase-I inhibition assay. A concentration-dependent decrease in the G-actin content was evident, with an optimal response at 1 ng/ml (final concn.) of insulin. A similar response was observed with glucagon (Fig. 1*b*), the optimal response being at 100 pg/ml (final concn.). The concentrations where optimal responses were obtained with both hormones were within the physiological range.

### *Effect of $T_4$ and $T_3$ on the actin state in hepatoma cells and polymorphonuclear leucocytes*

In contrast with the peptide hormones, which act through cell-surface receptors, $T_3$ and $T_4$ act through the nuclear receptors. Figs. 2(*a*) and 2(*b*) demonstrate the effect of $T_3$ and $T_4$, respectively, on the G-actin content in rat hepatoma cells. The optimal response was obtained at 1–10 $\mu$M with $T_4$ and 1–10 nM with $T_3$. Fig. 3 shows the effect of $T_3$ in polymorphonuclear leucocytes. An optimal response was seen at 10 nM, similar to the effect in hepatoma cells. Once again, the concentrations of $T_3$ and $T_4$ were in the physiological range.

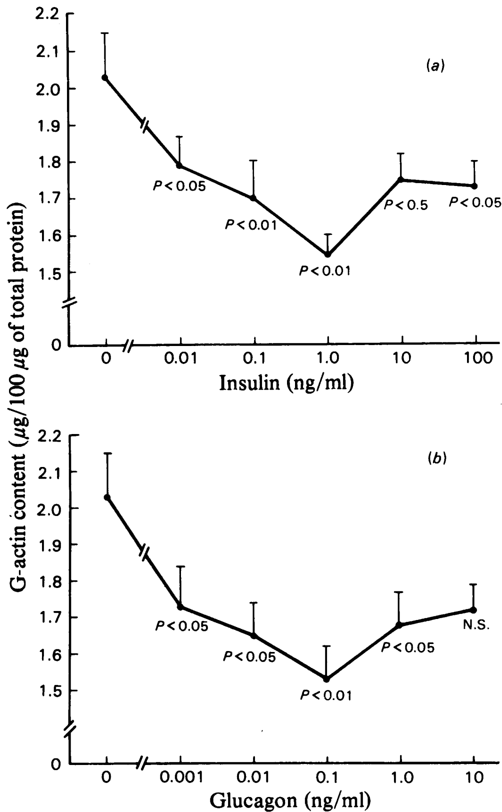


Fig. 1. *Effect of insulin (a) and glucagon (b) on the G-actin content in rat hepatoma cells*

Cell suspension (100 μl: approx. $3\times10^6$–$4\times10^6$ cells/ml) was incubated with various concentrations of insulin or glucagon for 5 min at 37°C. The cells were lysed and assayed for G-actin content within 1 min after lysis. Results are means ± S.E.M. (bars) for seven different experiments performed in duplicate. The $P$ values were obtained by paired $t$ test in comparison with control cells: N.S., not significant.

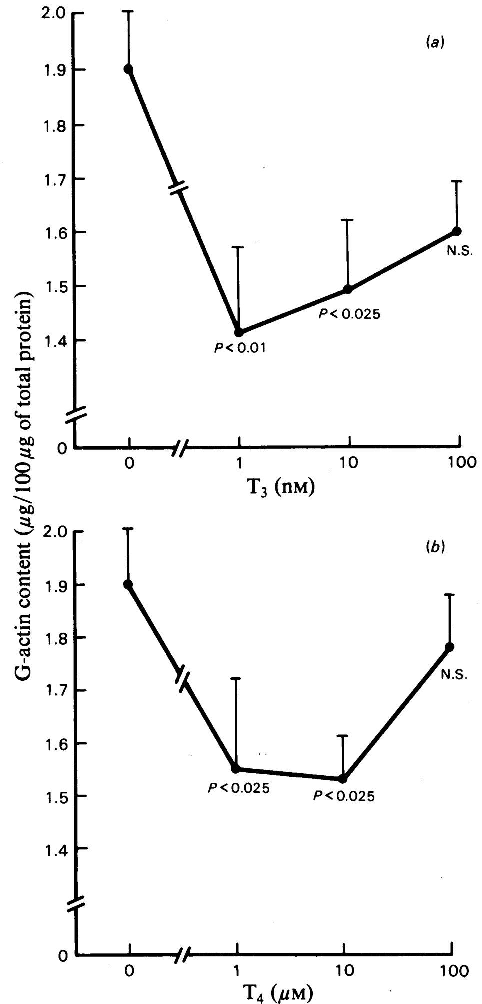


Fig. 2. *Effect of* $T_4$ *(a) and* $T_3$ *(b) on the G-actin content in rat hepatoma cells*

The experimental conditions are as described in Fig. 1. Results are means ± S.E.M. (bars) for four different experiments performed in duplicate. The $P$ values were obtained by paired $t$-test: N.S., not significant.

### *Effects of adrenergic agonists and antagonists on actin state in polymorphonuclear leucocytes*

Adrenergic stimulants are non-peptide molecules which act through cell-surface-receptor mechanisms. Figs. 4(*a*) and 4(*b*) demonstrate the effect of the α-agonist phenylephrine and the β-agonist isoprenaline on the G-actin content in human polymorphonuclear leucocytes. An optimum response was obtained at 0.1 μM (final concn.) with phenylephrine. The response to isoprenaline was relatively weak and showed a broad, flat, curve. The specificity of the phenylephrine action is demonstrated in Table 1. Pretreatment of polymorphonuclear leucocytes with phentolamine (0.1 mM) for 5 min inhibited the effect of phenylephrine. The effect was concentration-dependent, as no inhibitory effect was observed at 10 μM phentolamine.

## Discussion

Stimulation of hepatoma cells with insulin and glucagon and of polymorphonuclear leucocytes with adrenergic agonists resulted in a decrease in the amount of G-actin, as measured by the DNAase-I inhibition assay. The decrease in G-actin measured by this assay has been shown to

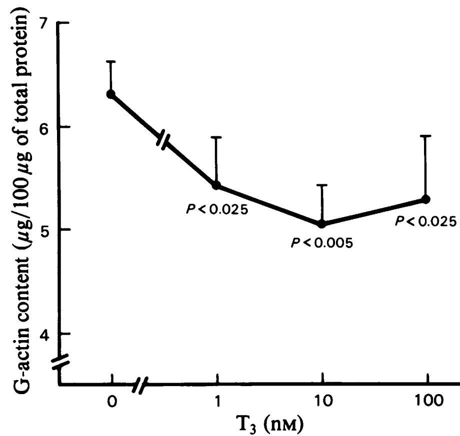


Fig. 3. *Effect of $T_3$ on the G-actin content in human polymorphonuclear cells*

Cell suspension (100μl; approx. $4\times10^6$–$5\times10^6$ cells/ml) was incubated with various concentrations of $T_3$ for 5min at 37°C. The cells were lysed and assayed for G-actin within 1min after lysis. Results are means ± S.E.M. for eight experiments performed in duplicate. The $P$ values were obtained by paired $t$-test.

Table 1. *Effect of phentolamine preincubation on phenylephrine-induced actin polymerization in polymorphonuclear leucocytes*

Control cells were incubated for 10min at 37°C without any drugs. The experimental cells were incubated for 5min at 37°C with or without phentolamine, and incubation continued for another 5min with phenylephrine. G-actin content was determined as described in the Materials and Methods section. Results are means ± S.D. ($n = 4$).

| Cells | G-actin (μg/100μg of total protein) |
|---|---|
| Control | 6.15 ± 0.73 |
| + Phenylephrine (0.1μM) | 4.37 ± 1.15 |
| + Phentolamine (0.1mM) + phenylephrine (0.1μM) | 6.62 ± 0.82 |
| + Phentolamine (10μM) + phenylephrine (0.1μM) | 4.55 ± 0.94 |

reflect the conversion of G-actin into polymeric actin (F-actin), as measured by electron microscopy (Carlsson *et al.*, 1979; Fechheimer & Zigmond, 1983) and 7-nitrobenz-2-oxadiazole–phallacidin labelling (Howard & Meyer, 1984; Wallace *et al.*, 1984). A variety of stimuli which result in cell activation induce actin polymerization, which led us to postulate that actin polymerization might be a universal intracellular event accompanying cell-surface receptor perturbation (Rao & Varani, 1982). In the present paper it is shown that both

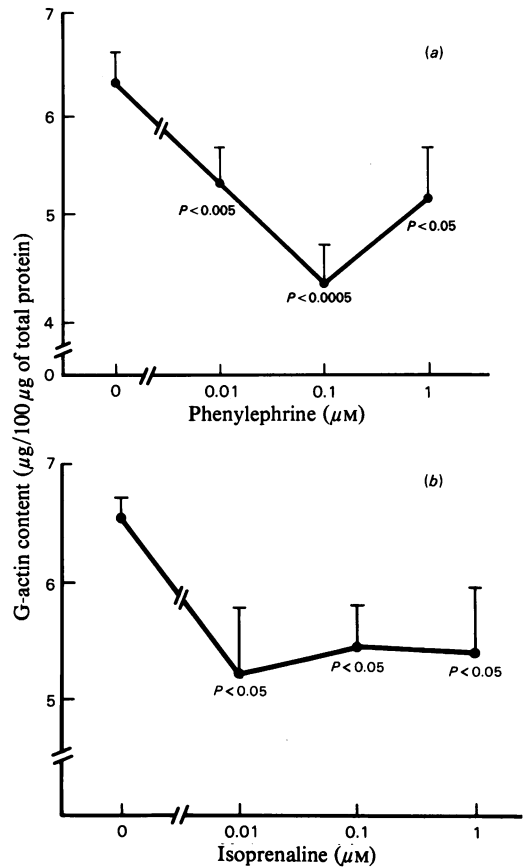


Fig. 4. *Effect of phenylephrine (a) and isoprenaline (b) on the G-actin content in human polymorphonuclear cells*

Experimental conditions were as described in Fig. 3. Results are means ± S.E.M. for eight (*a*) or five (*b*) experiments performed in duplicate.

groups of hormones, those whose effects are mediated through cell-surface receptors and also those that act through intracellular receptors, such as $T_4$ and $T_3$, induce actin polymerization. These observations extend the concept of actin polymerization as an event accompanying cellular responses to a variety of different hormonal stimuli.

Alterations in actin state are similar with hormones having opposing physiological functions. Insulin and glucagon and α- and β-adrenergic agents, which have opposing effects on adenylate cyclase (Limbird, 1983), evoke identical changes in actin state in the cells. This suggests that actin polymerization may be a common cellular event which occurs in response to a variety of stimuli acting at different cellular sites. The differences in physiological effects of hormones and other effectors might be attributable to quantitative differences in the variety of events, such as calcium fluxes and activation of protein

kinase C, that they elicit in a particular cell, with some events, such as actin polymerization, being common to a number of stimuli.

The change in actin state may not be limited to the cytoplasmic compartment. Thyroid hormones freely diffuse through the cell membrane and bind to nuclear receptors (Oppenheimer, 1979). Actin is the most abundant nuclear protein and appears equally distributed in the nucleus and the cytoplasm (DeRobertis *et al.*, 1978; LeStourgen, 1978). Therefore it is possible that the alterations in actin state observed result from the nuclear binding of thyroid hormones. Further studies might elucidate whether the hormone-induced actin changes occur in both nuclear and cytoplasmic compartments, or are confined to the cytoplasmic compartment for cell-surface-reactive agents and to the nuclear compartment with those agents that act through nuclear receptors.

Though it is well established that the microfilaments play an important role in a variety of cellular processes such as cell locomotion, cytoplasmic streaming and transport, secretion, phagocytosis and cytokinesis (Wessells *et al.*, 1971), the possibility that microfilaments may have a wider role in cellular physiology, including certain metabolic processes, is becoming apparent, through more-recent studies. Changes in actin state may be involved in processes as disparate as the transcription of chromosomes (Scheer *et al.*, 1984) and the regulation of energy metabolism. Histochemical observations of muscle reveal close association of the glycolytic enzymes at the site of actin filaments (Sigel & Pette, 1969), and it is established that actin is the main component in this interaction. A number of glycolytic enzymes have been shown to bind to reconstituted thin filaments, which are composed of F-actin, troponin and tropomyosin, under conditions of physiological ionic strength and protein concentration. Phosphofructokinase, pyruvate kinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate isomerase and lactate dehydrogenase have a large portion of their total activities bound to thin filaments (Arnold & Pette, 1968). Binding of glycolytic enzymes to thin filaments is known to activate several of these enzymes (Poglazov, 1983). Thyroid hormones, insulin and glucocorticoids regulate glyceraldehyde-3-phosphate dehydrogenase in cultured hepatocytes (Wilson & McMurray, 1981). Alterations in actin state in response to hormonal stimulation may be involved in this regulation. In fact, the concept of metabolic co-ordination by actin state has been elaborated in two reviews (Masters, 1981; Poglazov, 1983). The observations reported here support the theories which emphasize an important role for the cytoskeleton in hormonal action (Peters, 1956; Zor, 1983).

In summary, we have demonstrated that hormones induce a rapid decrease in G-actin in eukaryotic cells and suggest that actin polymerization may be an important common and essential event accompanying hormone-mediated changes in cell metabolism and growth.

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